UCL EASTMAN DENTAL INSTITUTE

ORAL MEDICINE UNIT

The GENVABO study

– genetic variants as biomarkers of jaw osteonecrosis associated with bisphosphonates: a large, multicentre genome-wide association study and detailed analyses of clinical phenotype

Pok Lam Fung

BDS (Hons) HKU, MSc Oral Medicine (Distinction) UCL

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University College London

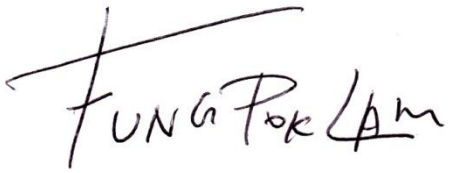
Oral Medicine Unit

UCL Eastman Dental Institute

2015

DECLARATION

I, Pok Lam Fung, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.



Pok Lam Fung

Oral Medicine Unit

UCL Eastman Dental Institute

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ABSTRACT

Osteonecrosis of the jaw (ONJ) is a potentially severe adverse effect of bisphosphonates. It can cause persistent pain and infection to the jawbones, and is currently considered incurable. ONJ occurs in a subset of individuals exposed to bisphosphonates (≤7%). Although a number of clinical risk factors, such as dentoalveolar surgery and dental infection, can increase the risk of ONJ development, there remains a number of patients who do not present with these clinical risk factors. Therefore, a genetic predisposition has been proposed.

Genome-wide association studies (GWAS), widely performed in pharmacogenomics and successful in other drug side effects, have also been attempted in bisphosphonates-associated ONJ. However, possibly due to small cohort sizes (≤30 cases), these studies failed to detect any significant genetic risk factors.

The aim of this thesis is to present the results of a large, multicentre GWAS, coupled with detailed analyses of clinical phenotype.

393 ONJ cases were recruited from 23 clinical centres worldwide. All cases were thoroughly phenotyped and adjudicated by specialist multidisciplinary teams. Random effects logistic regressions (Stata v12.1) were used for clinical risk factor analyses. All samples were genotyped using Illumina® Human1M Omni Express Beadchip (1,072,820 probes) and were compared with 2,554 genetically-matched population controls from publicly available sources. Genotype statistical analysis was performed in PLINK.

Risk factors including advanced age, longer bisphosphonates duration, other cancers and use of steroids were found statistically significant (p<0.05). With extreme phenotyping, i.e. non-surgery triggered ONJ cases versus the population controls, for the first time, a genome-wide significant single nucleotide polymorphism was identified: rs12440268 at *TJP1* gene (p=1.21E-8). Individuals positive for this marker were nearly three times more likely to develop ONJ than those negative for it (OR=2.66). *TJP1* encodes protein at the tight junctions, which maintain epithelial integrity. Its polymorphism may contribute to ONJ pathogenesis through impaired mucosal healing.

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ABBREVIATIONS

AAOMS	American Association of Oral and Maxillofacial Surgeons
ADR	Adverse Drug Reaction
Ale	Alendronate
ASBMR	American Society for Bone and Mineral Research
BNF	British National Formulary
BPs	Bisphosphonates
CNV	Copy Number Variation
CRF	Case Report Form
EMA	European Medicines Agency
FDA	Food and Drug Administration
FPPS	Farnesyl PyroPhosphate Synthase
GENVABO	GENetic VAriants as Biomarkers of jaw Osteonecrosis associated with bisphosphonates
GWAS	Genome-wide Association Study
Iba	Ibandronate
iSAEC	International Serious Adverse Event Consortium
MBC	Metastatic Breast Cancer
MHRA	Medicines and Healthcare products Regulatory Agency
MPC	Metastatic Prostate Cancer
ONJ	OsteoNecrosis of the Jaw
OR	Odds Ratio
Pam	Pamidronate
Ris	Risedronate
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
VAS	Visual Analogue Scale
VEGF	Vascular Endothelial Growth Factor
Zol	Zoledronate

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PUBLICATIONS, PRESENTATIONS & PUBLIC ENGAGEMENT

Journal papers

- Fung, P.L., Nicoletti, P., Shen, Y., Porter, S., and Fedele, S. (2015). Pharmacogenetics of Bisphosphonate-associated Osteonecrosis of the Jaw. Oral Maxillofac. Surg. Clin. N. Am.
- Fedele, S., Bedogni, G., Scoletta, M., Favia, G., Colella, G., Agrillo, A., Bettini, G., Di Fede, O., Oteri, G., Fusco, V., Gabriele, M., Ottolenghi, L., Valsecchi, S., Porter, S., Fung, P.L., Saia, G., Campisi, G., Bedogni, A. (2015). Up to a quarter of patients with osteonecrosis of the jaw associated with antiresorptive agents remain undiagnosed. Br. J. Oral Maxillofac. Surg. *53*, 13–17.
- Bedogni, A., Fedele, S., Bedogni, G., Scoletta, M., Favia, G., Colella, G., Agrillo, A., Bettini, G., Di Fede, O., Oteri, G., Fusco, V., Gabriele, M., Ottolenghi, L., Valsecchi, S., Porter, S., Petruzzi, M., Arduino, P., D'Amato, S., Ungari, C., Fung, P.L., Saia, G., Campisi, G. (2014). Staging of osteonecrosis of the jaw requires computed tomography for accurate definition of the extent of bony disease. Br. J. Oral Maxillofac. Surg. *52*, 603–608.

Invited lectures and talks

- The phossy jaw story by Polly Pok-Lam Fung, MSc, MPhil.

  Talk of the day, Royal College of Surgeons of England (RCS) Hunterian Museum; London, UK;

  Free talk to museum visitors, most Saturdays from Sep 2014 to Jan 2015.
- Genetic variants as biomarkers of jaw osteonecrosis associated with bisphosphonates (GENVABO) study: preliminary genome-wide association study (GWAS) result and phenotypic analyses on time to event and risk factors.

  10 May 2014 – Società Italiana di Patologia e Medicina Orale (SIPMO) Meeting; Alessandria, Italy.
- Genetic variants as biomarkers of jaw osteonecrosis associated with bisphosphonates.

  8 Nov 2013 – UCL Faculty of Medical Sciences Dean's Research Prize Event; London, UK.
- Pharmacogenomics of bisphosphonates: a genome wide study of genetic variants associated with jaw osteonecrosis.

  12 Oct 2013 – SIPMO 12th National, 2nd International Congress; Rome, Italy.

Conference papers

***Presenter**

Nicoletti, P., Fung, P.L.*, Porter, S., Fedele, S., GENVABO Consortium.

Genomewide association study of bisphosphonate-related jaw osteonecrosis (BONJ).

- British Pharmacological Society Annual Symposium Pharmacology 2014; London, UK.
- Wellcome Trust Genome Campus the Leena Peltonen School of Human Genomics 2014; Hinxton, Cambridge, UK.
- European Association of Oral Medicine (EAOM) 12th Biennial Congress 2014; Antalya, Turkey.

Fung, P.L.*, Petrie, A., Porter, S., Fedele, S., GENVABO Consortium.

Time to BONJ diagnosis: results from a large multicentre study.

- Joint Meeting of American Association of Oral Medicine, British Society of Oral Medicine (BSOM), EAOM and the Oral Medicine Academy of Australasia Frontiers in Oral Medicine 2014; Orlando, US.

Fung, P.L.*, Fedele, S., Manfredi, M., Vescovi, P., Merigo, E., Porter, S.

BONJ among osteoporosis patients: clinical phenotype, long-term behaviour and potential predictors.

- European Congress on Osteoporosis and Osteoarthritis 2013; Rome, Italy.
- National Institute for Health Research (NIHR) 7th Annual Trainee Meeting 2013; Leeds, UK.

Fung, P.L.*, Porter, S., Fedele, S., GENVABO Consortium.

Clinical phenotype and risk factors for bisphosphonate-associated osteonecrosis of the jaw.

- BSOM Annual Scientific Meeting 2013; Birmingham, UK.

Fung, P.L.*, Fedele, S., Manfredi, M., Vescovi, P., Merigo, E., Petrie, A., Porter, S.

Long-term behaviour of bisphosphonate-associated osteonecrosis of the jaw.

- EAOM 11th Biennial Congress 2012; Athens, Greece; 2nd Prize, Oral Presentation Award.
- NIHR 3rd Experimental Medicine Research Training Camp 2012; Ashridge, UK.
- BSOM Annual Scientific Meeting 2012; Liverpool, UK.

Public engagement

Phossy jaw research artwork exhibition.

Worked with postgraduates at Courtauld Institute of Art, designed and printed image.

- Online exhibition, Out of Our Heads, Art in medicine online, since Oct 2014. http://www.outofourheads.net/oooh/handler.php?id=724
- Exhibited at UK Universities Week, Jun 2014; Natural History Museum, London, UK.
- UCL Graduate School research images competition/exhibition 2013, Selected as 1 of 28 exhibits out of 330 entrants; UCL South Cloister, London, UK.

Phossy jaw research blog, http://phossyjaw.wordpress.com/

- Worked with historians and archivists, researched at British Library, Museum of London, RCS, Royal Society of Chemistry, UCL/UCLH archives
- Designed blog, wrote 22 posts
- >5,000 views by ~2,500 viewers from ~80 countries

Figure 1. Public engagement

From left to right: Phossy jaw research artwork exhibition at UK Universities Week, Natural History Museum. Exhibition at UCL South Cloister. Phossy jaw research blog.

CHAPTER 1

Introduction

Overview of ONJ features, clinical and genetic risk factors

1.1 Overview of jaw osteonecrosis

1.1.1 Introduction

Osteonecrosis of the jaw (ONJ) is a potentially severe adverse effect of bisphosphonate medications affecting the jawbones. Bisphosphonates are commonly used in managing bone diseases including osteoporosis, multiple myeloma and bone metastases from solid cancers.

Since its first report in 2003 (Marx, 2003), thousands of bisphosphonate-associated ONJ cases have been reported worldwide (Filleul et al., 2010). The disease is often painful and has a wide range of clinical features, which can lead to eating difficulties, speech impairment, facial disfigurement and overall significantly reduced quality of life (Miksad et al., 2011). To seek compensation for their drug side-effect, patients have attempted to bring pharmaceutical companies to court and the largest successful verdict to date involved USD 10.45 million (Barbara Davids and Helene Deutsch v. Novartis Pharmaceuticals Corporation, No. 06 431, U.S. District Court, Eastern District of New York, 11 July 2012).

Currently, there remains no consensus regarding the terminology and definition of ONJ, and little is known regarding its pathogenesis and the most effective management. In severe cases, patients may require jaw resection and reconstruction under general anaesthesia, in which surgical complications including death have been reported in some cases (Bedogni et al., 2011; de Boer et al., 2012). It is therefore a serious adverse drug reaction (ADR), defined as “an undesirable experience concerned with a particular drug and that leads to any of the following: death or life-threatening event,

hospitalisation, disability or permanent damage, congenital abnormality or birth defect" (Daly, 2012).

In the late 19th century, there was a similar disease known as the "phossy jaw", or phosphorus necrosis. It was an occupational disease amongst match factory workers who were exposed to toxic white phosphorus vapour. Once inhaled, white phosphorus reacts with water to form pyrophosphate, then it becomes bisphosphonate through combining with carbonic acid and amino acid. Therefore, "phossy jaw" and ONJ are similar diseases, both related to bisphosphonates, and they also share very similar clinical features (Marx, 2008).

1.1.2 Clinical features

1.1.2.1 Signs

ONJ affects the mandible in approximately 60% of cases, whereas about 30% develops in the maxilla. Few individuals, about 10%, have ONJ affecting both jaws (Woo et al., 2006).

It typically presents with brown or grey necrotic bone, exposed through the oral mucosa, gingiva or facial skin (Filleul et al., 2010). Some individuals present with the non-exposed ONJ variant, i.e. there is no frank bone exposure, but with the presence of unexplained jaw bone pain, fistula tract, bone or gingival swelling, not caused by dental or other bone diseases (Fedele et al., 2010) (Figure 2). The non-exposed variant had been neglected for some years until its first report by Junquera and Gallego, 2008. In 2015, Fedele et al., reported by far the largest case series of 192 patients with the non-exposed variant, representing one-fourth of all ONJ cases in their study cohort.

Both exposed and non-exposed ONJ are associated with a wide range of clinical manifestations including intra- and extra-oral fistulae, tooth mobility, maxillary sinusitis and pathological fracture of mandible; infection is common and is associated with soft tissue manifestations including erythema, bleeding, swelling and suppuration (Filleul et al., 2010).

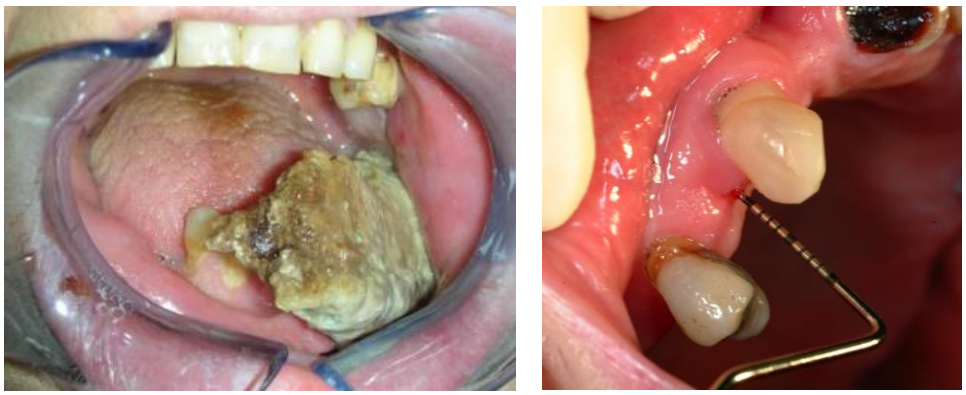
1.1.2.2 Symptoms

ONJ can be asymptomatic but pain is common – about 80% of patients report pain during the course of ONJ (Filleul et al., 2010; Otto et al., 2012). Patients may also complain of mobile teeth, gingival swelling, pus discharge, bad breath and paraesthesia (Vescovi et al., 2012).

1.1.2.3 Disease onset

There is a large variability in the time to onset of ONJ. It can vary according to the type of bisphosphonates. Studies reported that the average time from the start of bisphosphonates therapy to ONJ development is approximately 1.8 years for zoledronate and 4.6 years for alendronate (Palaska et al., 2009). As for ONJ onset event, about 60% were surgically-triggered, mainly dental extractions, while the rest were non-surgically-triggered (Vescovi et al., 2011).

Figure 2. Exposed and non-exposed ONJ



Left: Exposed ONJ of the left mandible, extensive exposure of brown or grey necrotic bone. Right: Non-exposed ONJ of the right maxilla, a small sinus tract detected clinically by a periodontal probe.

1.1.3 Diagnosis, definition and disease staging

Diagnosis of ONJ is usually made through history-taking and clinical examination (Ruggiero and Mehrotra, 2009; McLeod et al., 2012; Khan et al., 2015). Biopsy is not mandatory but can be useful for excluding other jawbone disorders, such as metastasis (Khosla et al., 2007; Arrain and Masud, 2011; Bhatt et al., 2014). Although there is no specific imaging features for ONJ, it remains helpful in differential diagnosis and disease staging (Khan et al., 2015).

Before 2014, ONJ case definition proposed in 2009 by the American Association of Oral and Maxillofacial Surgeons (AAOMS) had been most widely accepted (Ruggiero et al., 2009; McLeod et al., 2011, 2012). There were also the American Society for Bone and Mineral Research (ASBMR) definition, the British Dental Association and the Scottish Dental Clinical Effectiveness Programme definitions, all very similar to the one by AAOMS 2009 (Arrain and Masud, 2011) (Table 1).

However, the 2009 definition only included the exposed variant of ONJ. An increasing number of authors have been calling for its revision to include the non-exposed variant (Colella et al., 2009; Yarom et al., 2010; Bedogni et al., 2012; Fedele et al., 2015). In 2014, the AAOMS definition was revised and “bone that can be probed through a fistula” was finally included (Ruggiero et al., 2014).

A number of ONJ staging systems have been introduced and the AAOMS 2009 staging system had also been widely used (McLeod et al., 2012) (Table 2). However, it failed to classify, for instance, non-exposed ONJ cases with jaw fracture or extraoral fistula (Bagan et al., 2012). In the new AAOMS 2014

staging system, non-exposed cases were also properly incorporated (Ruggiero et al., 2014) (Table 3).

Table 1. ONJ definitions

Year	Organisation	Definition	Reference
2007, 2009	AAOMS	• Current or previous treatment with a bisphosphonate • Exposed, necrotic bone in the maxillofacial region that has persisted for more than 8 weeks • No history of radiation therapy to the jaws	(AAOMS 2007), (Ruggiero et al., 2009)
2006	Australian and New Zealand Bone and Mineral Society, Osteoporosis Australia, Medical Oncology Group of Australia, and the Australian Dental Association	An area of exposed bone that persists for more than 6 weeks	(Sambrook et al., 2006)
2007	ASBMR	• Confirmed case: same as AAOMS 2007/09 • Suspected case: same as AAOMS 2007/09 except exposed bone has been present for less than 8 weeks	(Khosla et al., 2007)
2012	Expert Panel of the Italian Society for Maxillofacial Surgery (SICMF) and the Italian Society of Oral Pathology and Medicine (SIPMO) on Bisphosphonate-Related Osteonecrosis of the Jaws	An adverse drug reaction described as the progressive destruction and death of bone that affects the mandible or maxilla of patients exposed to the treatment with nitrogen-containing bisphosphonates, in the absence of a previous radiation treatment	(Bedogni et al., 2012)
2014	AAOMS	• Current or previous treatment with antiresorptive or antiangiogenic agents • Exposed bone or bone that can be probed through an intraoral or extraoral fistula(e) in the maxillofacial region that has persisted for more than 8 weeks • No history of radiation therapy to the jaws or obvious metastatic disease to the jaws	(Ruggiero et al., 2014)

Table 2. ONJ staging systems

Year	Organisation	Staging system			Reference
		Diagnosis	No. of stages	Inclusion of non-exposed ONJ	
2006	AAOMS	Clinical examination	3	No	(AAOMS 2007)
2007	/	Clinical examination, radiographs, computed tomography (CT), magnetic resonance imaging (MRI), biopsy	6	Yes	(McMahon et al., 2007)
2009	AAOMS	Clinical examination; radiographs for non-exposed variant	4	Yes	(Ruggiero et al., 2009)
2012	SICMF and SIPMO on ONJ	Clinical examination, CT	3	Yes	(Bedogni et al., 2012)
2014	AAOMS	Clinical and radiographic examinations	4	Yes	(Ruggiero et al., 2014)

Table 3. AAOMS staging (Ruggiero et al., 2014)

Stage	Description
At Risk Category	No apparent necrotic bone in patients who have been treated with either oral or IV bisphosphonates
Stage 0	No clinical evidence of necrotic bone, but non-specific clinical findings, <u>radiographic changes</u> and symptoms
Stage 1	Exposed and necrotic bone, <u>or fistulae that probes to bone</u>, in patients who are asymptomatic and have no evidence of infection
Stage 2	Exposed and necrotic bone, <u>or fistulae that probes to bone</u>, associated with infection as evidenced by pain and erythema in the region of the exposed bone with or without purulent drainage
Stage 3	Exposed and necrotic bone <u>or a fistula that probes to bone</u> in patients with pain, infection, and one or more of the following: exposed and necrotic bone extending beyond the region of alveolar bone,(i.e., inferior border and ramus in the mandible, maxillary sinus and zygoma in the maxilla) resulting in pathologic fracture, extra-oral fistula, oral antral/oral nasal communication, or osteolysis extending to the inferior border of the mandible of sinus floor

Underlined: updates in 2014 as compared to 2009 staging

1.1.4 Risk reduction and management

The objectives of most risk reduction strategies are to improve dental hygiene and minimise surgical trauma from tooth extraction and implant surgery (Khan et al., 2015). However, these strategies are mostly based on expert opinion and are not supported by robust evidence (Fedele et al., 2009).

Regarding treatment, ONJ is considered incurable as bone necrosis cannot be reversed (Landis et al., 2006). In addition, bisphosphonates have a very long half-life and remain in the jawbone for many years (Kimmel, 2007). Most management strategies aim at pain and infection control, consisting mainly of symptomatic treatment and minimally invasive surgery (McLeod et al., 2011). AAOMS and the Canadian Association of Oral and Maxillofacial Surgeons recommended treatment according to ONJ staging, ranging from optimal oral hygiene, topical antibiotic rinse and systemic antibiotics, to debridement and major resection and reconstruction surgery (Khan et al., 2015); similar recommendation has also been suggested by the ASBMR (Khosla et al., 2007).

1.1.5 Epidemiology

Data on ONJ epidemiology remain unclear, mainly due to small cohorts and heterogeneous study designs (Ruggiero, 2011; Campisi et al., 2014). In 2012, Kühl et al. reviewed nearly 700 publications and reported a wide incidence range of 0.0 to 27.5%. The average incidence amongst patients who were on intravenous bisphosphonates was 7% and that amongst oral bisphosphonates patients was 0.01%.

1.2 Causes of ONJ in association with bisphosphonates

1.2.1 Introduction

There is little doubt that individuals exposed to bisphosphonates are at risk of developing ONJ (Abrahamsen, 2010; Barasch et al., 2011; Pautke et al., 2012). Currently, ONJ is recognised as one of the major ADR of bisphosphonates by drug agencies around the world, including the European Medicines Agency (EMA), Medicines and Healthcare products Regulatory Agency (MHRA), British National Formulary (BNF) and the US Food and Drug Administration (FDA).

1.2.2 Bisphosphonates

1.2.2.1 Biochemistry

Bisphosphonates (BPs) are pyrophosphate analogues and all have a strong P-C-P bond in its core, making BPs resistant to enzymatic reaction; the phosphate groups and side chains enable BPs to bind with hydroxyapatite crystals, which explains its high affinity for bone (Russell, 2011). BPs refer to a group of drugs, which can be classified by chemical structure or route of administration (Table 4). Nitrogen-containing and non-nitrogen-containing BPs differ in their chemical structure and mechanism of action. In general, nitrogen-containing BPs are of higher potency (Fleisch, 1998).

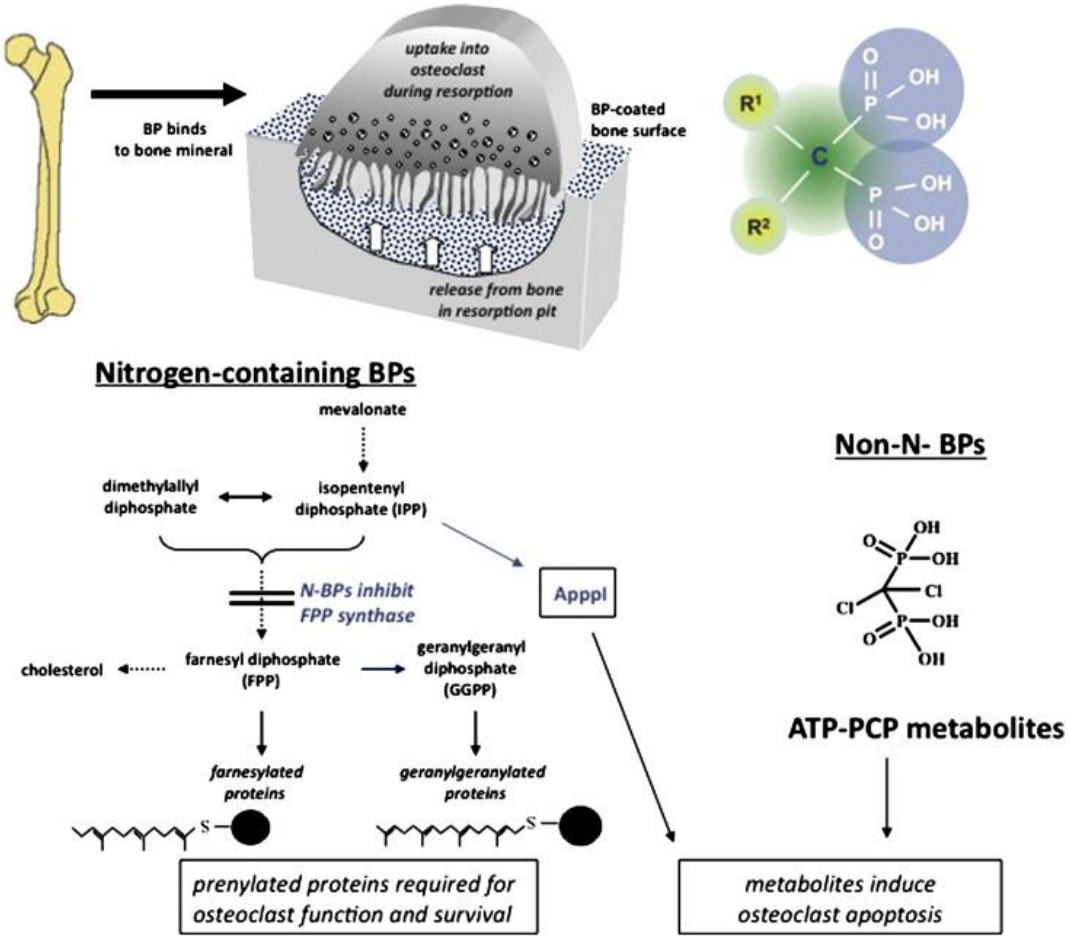
Table 4. Classification of BPs

Chemical structure		Route of administration	
Nitrogen-containing	**Non-nitrogen-containing**	**Intravenous**	**Oral**
Alendronate	**Clodronate**	**Ibandronate** (Bondronat, Bonviva)	**Alendronate** (Fosamax, Fosavance)
Risedronate	**Etidronate**	**Disodium Pamidronate** (Aredia)	**Sodium Clodronate** (Bonefos, Loron)
Ibandronate	**Tiludronate**	**Zoledronate** (Aclasta, Zometa)	**Disodium Etidronate** (Didronel, Didronel PMO)
Pamidronate			**Ibandronate** (Bondronat, Bonviva)
Zoledronate			**Risedronate Sodium** (Actonel, Actonel Once a Week)
			Disodium Tiludronate (Skelid)

1.2.2.2 Pharmacology and mechanism of action

Bioavailability of oral BPs is poor, about 0.7%. Subsequent to absorption at the gastrointestinal tract, BPs are then taken up primarily by bone tissue and retained for a long time, and ultimately excreted unchanged in urine (Rodan et al., 2004; Cremers and Papapoulos, 2011). After binding to bone, BPs are internalised into osteoclast by endocytosis. Nitrogen-containing BPs inhibit farnesyl pyrophosphate synthase (FPPS), a key enzyme of the mevalonate pathway; this (i) prevents prenylation of GTPase proteins which are essential for osteoclast function and survival, and (ii) causes accumulation of isopentenyl diphosphate which can induce osteoclast apoptosis. Non-nitrogen-containing BPs are incorporated into ATP analogue, which can also induce osteoclast apoptosis (Kavanagh et al., 2006; Rondeau et al., 2006; Thompson et al., 2006; Rogers et al., 2011) (Figure 3).

Figure 3. Mechanism of action of BPs; Courtesy: Russell 2011.



In summary, BPs act mainly by inhibiting osteoclast and bone resorption; they may also have effects on other cells such as osteoblast, osteoclast precursor, tumour cell and macrophage (Russell et al. 2007).

1.2.2.3 Indications

Given their antiresorptive property, BPs are widely used in managing bone malignancy, osteoporosis and other bone diseases.

BPs are helpful in managing multiple myeloma and metastatic bone diseases (Lipton et al., 2003; Rosen et al., 2004; Saad et al., 2004; Coleman and McCloskey, 2011). The 2012 Cochrane review concluded that BPs prevent pathological vertebral fractures, skeletal-related events and pain, and improve overall survival of multiple myeloma (Mhaskar et al., 2012). According to two

other Cochrane reviews, BPs also reduce skeletal-related events and pain of metastatic breast and prostate cancers (Yuen et al., 2006; Wong et al., 2012).

In the UK, the National Institute for Health and Clinical Excellence (NICE) recommends the use of alendronate, etidronate and risedronate as first-line drugs for preventing fragility fracture in postmenopausal osteoporosis women.

In the US, as many as one in seven postmenopausal women have been treated with BPs and over 150 million prescriptions were dispensed between 2005 and 2009 (Black et al., 2012; Whitaker et al., 2012).

Evidence also suggests that BPs can control Paget's disease of bone, the second most common metabolic bone disease after osteoporosis (Reid and Hosking, 2011). Another Cochrane report concluded that BPs increase bone mineral density in osteogenesis imperfecta patients (Dwan et al., 2014). BPs can also manage other bone disorders including periprosthetic bone loss, fibrous dysplasia and calcinosis in juvenile dermatomyositis (Silverman, 2011).

1.2.2.4 Adverse effects

In addition to ONJ, other adverse effects of BPs include oesophageal ulceration, renal toxicity, atrial fibrillation and atypical femoral fracture, according to MHRA's safety information. Acute-phase reaction is common and occurs in about 40% of patients on nitrogen-containing BPs (Olson and Van Poznak, 2007). Other reported adverse effects include oesophageal cancer, ocular inflammation and musculoskeletal pain (Pazianas and Abrahamsen, 2011).

1.2.3 ONJ pathogenesis

To date, the exact pathogenesis of BPs-associated ONJ remains largely unknown. However, several hypotheses have been proposed and ONJ is considered a multifactorial disease (Landesberg et al., 2011; Kumar and Sinha, 2013).

1.2.3.1 Infection

Infection is common in ONJ (Katsarelis et al., 2015). Histopathologically, inflammatory infiltrate and bacterial colonisation were found in about 80% of the necrotic bone samples, with *Actinomyces, Streptococcus* being the most frequently reported (Hinson et al., 2014). Therefore, topical antibiotic rinse and systemic antibiotics are often prescribed in managing ONJ (Khan et al., 2015). However, it remains unclear whether infection initiates ONJ or it occurs as a secondary event after ONJ develops.

1.2.3.2 Impaired wound healing

Clinical studies reported that BPs can delay healing and its discontinuation can result in faster resolution of ONJ symptoms (Hasegawa et al., 2013; Hinson et al., 2015). In vitro studies also showed that BPs are toxic to soft tissue and inhibit oral mucosal cell proliferation and wound healing (Landesberg et al., 2008; Kumar and Sinha, 2013). However, this hypothesis is mainly relevant to cases presenting with an open wound, mostly caused by an invasive procedure, such as tooth extraction and implant surgery. As for cases with no history of dentoalveolar surgery, also known as spontaneous ONJ, the same hypothesis fails to explain their ONJ development. Spontaneous ONJ cases, first reported 10 years ago (Marx et al., 2005), are not uncommon and have been suggested to account for nearly 40% of all

cases in a large study of more than 500 ONJ cases (Vescovi et al., 2011).

1.2.3.3 Impaired angiogenesis

It is suggested that BPs can reduce angiogenesis through inhibition of vascular endothelial growth factor (VEGF) (Wood et al., 2002; Kobayashi et al., 2010; Vincenzi et al., 2012). A reduction in the number of blood vessels (Aguirre et al., 2010, 2012), as well as reduction in their quality including thin-walled, dilated, less connected and less ordered vessels, in association with BPs, have been reported (Favia et al., 2009; Guevarra et al., 2015). However, ONJ cases with intact and normal vascularity in both the alveolar bone (Hellstein and Marek, 2005) and the overlaying soft tissue have also been reported (Scheller et al., 2011; Wehrhan et al., 2011). Therefore, whether or not impaired angiogenesis contributes to ONJ development remains unclear.

1.2.3.4 Suppressed bone turnover

Most cases of osteonecrosis develop in the maxilla and/or the mandible. However, a handful of osteonecrosis cases in the auditory canal have also been reported (Salzman et al., 2013; Thorsteinsson et al., 2014). Allen, 2011 suggested that the jawbones are more likely to be affected because, compared to other skeleton sites, they have a relatively high remodelling rate, hence more susceptible to BPs' osteoclast inhibition and bone turnover suppression.

1.2.4 Clinical risk factors

1.2.4.1 BPs-related factors

Studies in beagle dogs confirmed the association of the degree of bone turnover suppression with BPs potency, binding affinity and cumulative dose (Allen and Burr, 2008; Allen et al., 2010). Clinical findings do agree with these experimental results as ONJ incidence and the number of cases are both

higher amongst patients exposed to high potency BPs, such as zoledronate, than in patients treated with alendronate, which is about 10-100 times less potent (Filleul et al., 2010; Kühl et al., 2012). Moreover, BPs duration and cumulative dose seem to be consistent and important risk factors for ONJ development (Fehm et al., 2009; Then et al., 2012; Campisi et al., 2014).

1.2.4.2 Systemic factors

1.2.4.2.1 Diabetes

The relationship between diabetes and ONJ has been inconclusive. There were studies reporting higher ONJ incidence amongst diabetic patients (Thumbigere-Math et al., 2012; Watters et al., 2012). In contrast, similar ONJ occurrence, regardless of the presence or absence of diabetes, has also been observed (Iwamoto et al., 2011). On the other hand, studies on the effect of diabetes phenotype upon ONJ development, such as diabetes severity or disease duration remain uncommon. Although it has been suggested that diabetes is associated with microvascular ischemia, reduced bone remodelling, increased inflammation and risk of infection, the exact pathogenesis of how diabetes induces ONJ remains unclear (Peer and Khamaisi, 2015).

1.2.4.2.2 Smoking

Whether or not smoking increases ONJ risk is unclear. A positive association of ONJ with smoking has been supported by the following studies: Wessel et al., 2008; Katz et al., 2011; Thumbigere-Math et al., 2012; Watters et al., 2012, but not supported by Kyrgidis et al., 2008, Vahtsevanos et al., 2009, a case-control study of over 1,600 patients, and Tsao et al., 2013.

1.2.4.2.3 Concomitant medications

It has been suggested that the concomitant use of antiangiogenic agents

constitutes an ONJ risk factor (Troeltzsch et al., 2012). There were also ONJ cases associated with bevacizumab and sunitinib *per se*, in the absence of BPs therapy (Estilo et al., 2008; Greuter et al., 2008; Serra et al., 2009; Koch et al., 2011; Bettini et al., 2012; Brunamonti Binello et al., 2012; Dişel et al., 2012; Fleissig et al., 2012; Hopp et al., 2012; Nicolatou-Galitis et al., 2012; Santos-Silva et al., 2013; Sivolella et al., 2013). However, whether or not these agents increase ONJ risk amongst BPs users remains controversial (Aragon-Ching et al., 2009; Christodoulou et al., 2009; Lazarovici et al., 2009; Guarneri et al., 2010; Francini et al., 2011).

Thalidomide, another commonly prescribed medication with antiangiogenic effects, has never been reported to cause ONJ in the absence of BPs therapy; evidence that concomitant thalidomide increases ONJ risk amongst BPs users is also weak (Zervas et al., 2006; Boonyapakorn et al., 2008).

It also remains unclear whether corticosteroids, another commonly prescribed concomitant medication in the BPs population, increase the risk of ONJ development (Lazarovici et al., 2009; Kos et al., 2010; Otto et al., 2012; Taylor et al., 2013).

Of note, bevacizumab and sunitinib are the only concomitant medications that are suggested to increase the risk on ONJ by the MHRA and BNF.

1.2.4.3 Local factors

Dentoalveolar surgery has always been considered a strong risk factor for ONJ, which seems to be supported by sound and consistent data (Campisi et al., 2014). However, a recent review concluded that the prevalence of ONJ amongst cancer patients following dental extraction was only 3.25% (Utreja et

al., 2013).

Periodontal disease has also been suggested to be an ONJ risk factor (Campisi et al., 2014). However, diagnosis of periodontal disease can be complicated in non-dental settings, as it requires probing depth, bleeding and plaque indices assessment. This has therefore only been supported by a limited number of small-scale studies (Thumbigere-Math et al., 2013).

1.2.5 Genetic variants

The occurrence of ONJ in a subset of individuals exposed to BPs suggests that its development may be related to genetic predispositions. In the past seven years, there have been a number of small pharmacogenetic studies on ONJ (Sarasquete et al., 2008; English et al., 2010; Katz et al., 2011; Arduino et al., 2011; Di Martino et al., 2011; Marini et al., 2011; Such et al., 2011; Nicoletti et al., 2012; La Ferla et al., 2012; Balla et al., 2012; Stockmann et al., 2013). By definition, pharmacogenetics is the study of how genetic differences influence the variability in patients' responses to drugs, including toxicity (Roses, 2000). It comprises mainly genome-wide association studies (GWAS) and candidate gene studies (Daly, 2010).

1.2.5.1 GWAS on ONJ

GWAS is a comprehensive research approach that is useful for investigating both complex disease and drug response including ADR. Typically, a GWAS screens millions of single nucleotide polymorphisms (SNPs) across the entire genome, in which a SNP refers to a single-base difference in DNA sequence in at least 1% of the general population (Daly, 2012). Most GWAS is of case-control design and a SNP is identified as a risk factor when its minor allele

frequency amongst the cases is significantly higher than in the controls.

Because GWAS tests millions of SNPs, it is possible that some variants have high frequency and small *p*-values simply by chance. To avoid any false positives, a stringent statistical correction for multiple comparisons is commonly required, which is known as Bonferroni correction. Instead of the usual $p<0.05$, the significance level for GWAS is calculated as 0.05 divided by roughly 1 million SNPs, i.e. $p<5E-08$ (Daly, 2012).

To date, two GWAS have been conducted on BPs-associated ONJ and relevant results are summarised in Table 5. The first GWAS, also the first pharmacogenetic study on ONJ, was published in 2008 by a Spanish team (Sarasquete et al., 2008). They studied 87 pamidronate-treated multiple myeloma patients, who were of Spanish descent, of whom 22 had developed ONJ. These cases were compared with 65 drug-exposed controls who had not developed ONJ after a median follow-up of 64 months. A total of 500,568 SNPs were screened and rs1934951 in *CYP2C8* was found to be most significant, although it did not reach genome-wide threshold of significance (OR=12.75, 95% CI 3.7 to 43.5, $p=1.07E-06$). This study suggested that individuals with this SNP had nearly 13 times greater odds of developing ONJ than those without it. Though not directly affecting BPs' metabolism, *CYP2C8* is known to be involved in osteoclast inhibition, osteoblast differentiation, and regulation of vascular tone, which may contribute to ONJ development (Sarasquete et al., 2009).

The second GWAS was published in 2012 and compared 30 zoledronate-treated breast cancer patients who developed ONJ with 17 drug-exposed

controls and 1,726 population controls (Nicoletti et al., 2012). The participants were of European descent. Compared to the previous GWAS, 731,442 SNPs were screened. Standard imputation was performed to enrich the genotype dataset, and an imputed SNP, rs17024608 in *RBMS3*, was found to be associated with ONJ, with borderline genome-wide significance (OR=5.8, 95% CI 3.0 to 11.0, *p*=7.47E-08). The rs17024608 carriers have approximately six times higher odds of developing ONJ than the non-carriers. *RBMS3* is a gene involved in bone turnover and has been found to associate with decreased bone mass and osteoporotic fracture (Nicoletti et al., 2012). Of note, *CYP2C8* was not found a significant risk factor for ONJ in this cohort of breast cancer patients.

1.2.5.2 Candidate gene studies on ONJ

Similar to GWAS, candidate gene studies often have a case-control design (Daly and Day, 2001). In general they focus on potentially biologically relevant genes. For ADR, most of the established and high risk genetic risk factors are relevant to drug metabolism or transporters genes (Daly, 2013). In contrast to GWAS, candidate gene studies screen much fewer variants and do not represent a hypothesis-free approach (Tabor et al., 2002). They are also prone to methodological weaknesses as they typically have small cohort size, no Bonferroni correction for the *p*-value, and often do not correct for the ethnicity of the cohort. Therefore, it has been suggested that candidate gene design is more suitable for replication studies (Kraft et al., 2009).

A total of nine candidate gene studies on BPs-associated ONJ were published between 2010 and 2013, including both replication and discovery gene studies, summarised in Table 6 and Table 7 .

1.2.5.2.1 Replication candidate gene studies

Four candidate gene studies attempted to replicate the results of the Spanish GWAS amongst pamidronate-treated multiple myeloma patients (Sarasquete et al., 2008), through investigating the possible association between rs1934951 in *CYP2C8* and ONJ in their respective independent cohorts (Table 6). All studies failed to confirm that this variant is significant (p>0.05). Paradoxically, English et al., 2010 and Katz et al., 2011 reported a protective OR for this variant. These contradicting results are likely to be related to the design of the replication studies, which failed to investigate populations ethnically and phenotypically similar to that of the original discovery study.

In contrast to the first GWAS, none of the four studies included individuals of Spanish descent, although their cohorts consisted mainly of White or Caucasian participants; African Americans were also inappropriately included (Arduino et al., 2011; Katz et al., 2011). Also, all four cohorts were predominantly exposed to zoledronate instead of pamidronate. Furthermore, only two replication studies focused on multiple myeloma patients (Katz et al., 2011; Such et al., 2011) whereas one recruited individuals with metastatic prostate cancer (English et al., 2010), and another included individuals with osteoporosis and a wide range of malignant disorders (Balla et al., 2012).

A recent meta-analysis attempted data pooling from the four candidate gene replication studies and the discovery Spanish GWAS (Zhong et al., 2013). They reported that rs1934951 in *CYP2C8* is not associated with ONJ across the entire merged population (OR=2.05, 95% CI 0.67 to 6.29, p=0.2). However, it might still be associated with ONJ development in multiple myeloma patients (OR=5.77, 95% CI 1.21 to 27.63, p=0.03).

Of note, there remains no published replication of rs17024608 in *RBMS3* identified in Nicoletti et al., 2012.

1.2.5.2.2 Discovery candidate gene studies

Six discovery candidate gene studies investigated variants in genes other than *CYP2C8* and are summarised in Table 7. These studies analysed the separate and combined effects of variants located in several genes, which had been chosen as they may relate to BPs metabolism and/or ONJ pathogenesis, e.g. bone turnover. Most of them screened only a small number of variants, and had relatively small cohorts, and are therefore susceptible to methodological limitations such as inadequate power. Of note, none of the SNPs tested reached the genome-wide significance level of p<5E-08.

The largest discovery candidate gene study in the literature compared 94 ONJ cases with 110 ethnicity matched BPs-exposed controls (Stockmann et al., 2013). The cohort included individuals with malignant disorders, including multiple myeloma, breast and prostate cancer, who were exposed mainly to zoledronate or pamidronate. The study hypothesis was that ONJ susceptibility might be linked to the major histocompatibility complex (*MHC*) class II system, which encodes *HLA* class II alleles. *HLA* alleles are major genetic risk factors for ADRs and are also associated with the adaptive immune system and infection, which in the case of ONJ may be related to the antigen-presenting function of osteoclasts and increased infection and/or inflammation (Landesberg et al., 2011). According to the significance threshold set by the study, two independent *HLA* haplotypes, DRB1*01/DRB1*15 and DQB1*05:01/DQB1*06:02, were found significant (OR>2, uncorrected $p \leqslant 0.05$). Moreover, the association appeared to be stronger when more than one

haplotype were considered together (OR=3, corrected *p*=0.0003) (Stockmann et al., 2013).

An Italian study by Arduino et al., recruited 30 women with breast cancer or multiple myeloma who developed zoledronate-induced ONJ cases, 30 drug, gender, disease and ethnicity-matched controls without ONJ, as well as 125 healthy controls. Candidate gene of this study was *VEGF*, which had been previously reported to be associated with avascular osteonecrosis at the femoral head (Kim et al., 2008; Lee et al., 2012). No statistically significant association was found for any of the three studied SNPs, -634 G>C, +936 C>T, and -2578 C>A (*p*>0.05). However, the haplotype determined by rs2010963 and rs699947 was found to be significant (corrected *p*=0.02) (Arduino et al., 2011).

Another Italian study by La Ferla et al., studied 30 zoledronate-induced ONJ cases and 53 zoledronate-exposed controls with multiple myeloma, breast and prostate cancer. Participants were tested for polymorphisms in aromatase and oestrogen receptor, which were selected because of their reported effects upon bone mineral density and remodelling. Results showed that rs10046 (g.132810C>T), a polymorphism in gene *CYP19A1*, was more prevalent amongst ONJ cases (OR=2.83, *p*=0.04) (La Ferla et al., 2012).

Marini et al., recruited 64 Italian patients with multiple myeloma, breast and prostate cancer who received zoledronate, 34 of whom developed ONJ. They studied polymorphism rs2297480 in gene *FDPS* (farnesyl pyrophosphate synthase, a key enzyme of the mevalonate pathway of osteoclasts), which was found to be significantly associated with ONJ, although not genome-wide

significant (p=0.03). This study represents the first attempt to investigate a candidate gene directly involved in BPs mechanism of action (Marini et al., 2011).

Katz et al., recruited multiple myeloma patients only, including 12 ONJ cases and 66 controls, who were managed with zoledronate and/or pamidronate. In addition to gene *CYP2C8*, six other candidate genes were studied based on their potential roles in osteoclast genesis and differentiation, bone resorption and bone mineral density. The results showed that all candidate genes *per se* had no effects on ONJ, although a combined genotype of *COL1A1*, *RANK*, *MMP2*, *OPG* and *OPN* was significantly associated with ONJ development (OR=1.2, 95% CI 1.8 to 69.9, p=0.0097) (Katz et al., 2011).

Di Martino et al., studied 1,936 SNPs relevant to 225 genes associated with drug metabolism, disposition and transport in nine multiple myeloma zoledronate-treated patients with ONJ and 10 matched controls. The authors claimed that using a platform that interrogates only highly selective SNPs has the advantage of avoiding any extremely high number of comparisons, and therefore the need for statistical corrections and large patient cohorts. As a consequence, the study adopted an uncorrected significance level of p<0.05 and reported that variants in four genes, *PPARG*, *ABP1*, *CHST11* and *CROT*, were statistically significant. However, since nearly 2,000 SNPs were screened, Bonferroni correction was required and the significance threshold should be approximately 2.5E-5 instead, i.e. 0.05 divided by 1,936 (Rice et al., 2008). This would mean that, in fact, no SNPs reached the corrected significance threshold. Nonetheless, on the basis of uncorrected results, patients with rs1152003, top SNP in *PPARG*, had over 30 times higher odds

of developing ONJ (OR=31.5, 95% CI 2.35 to 422.32, *p*=0.0055). Of note, *PPARG* has also been associated with bone remodelling, bone mass density, as well as angiogenesis (Di Martino et al., 2011).

1.3 Summary

Little robust information is available regarding the aetiopathogenesis of BPs-associated ONJ and it is unclear why it develops in a subset of patients. A number of clinical risk factors have been suggested; however, relevant literature lacks robustness and consistency, and in most instances ONJ remains an unpredictable ADR.

There is likely a genetic predisposition for ONJ; however, previous pharmacogenetic studies on ONJ were of small cohort sizes and no genome-wide significant variants have been identified.

Table 5. Summary of GWAS on ONJ

Year	Population	Underlying disease	BPs type	Case *n*	Control *n*	Genotyping	SNP	Gene	Chr	*p*-value	OR [95% CI]	Ref
2008	Spanish	Multiple myeloma	Majority on Pamidronate Zoledronate	22	65 BPs controls	Affymetrix GeneChip Mapping 500K set 500,568 SNPs screened	rs1934951	*CYP2C8*	10	1.07E-06	12.75 [3.7-43.5]	(Sarasquete et al., 2008)
							rs1934980	*CYP2C8*	10	4.23E-06	13.88 [4.0-46.7]	
							rs1341162	*CYP2C8*	10	6.22E-06	13.27 [3.5-49.9]	
							rs17110453	*CYP2C8*	10	2.15E-05	10.2 [3.2-32.1]	
2012	North-western, southern, eastern European descent	Osteoporosis Breast cancer	Majority on Zoledronate	30	17 BPs controls 1,726 population controls	Illumina Human Omni Express 12v1.0 chip 731, 442 SNPs analysed	rs17024608	*RBMS3*	3	7.47E-08	5.8 [3.0-11.0]	(Nicoletti et al., 2012)
							rs5768434	*FAM19A5*	22	1.17E-07	12.6 [4.9-32.2]	
							rs11064477	*PHB2*	12	5.16E-07	21.7 [6.5-71.9]	
							12–7016684	*C1S*	12	5.85E-07	21.1 [6.4-69.8]	
							8–58133986	*IMPAD1*	8	3.10E-06	7.3 [3.1-16.9]	
							rs1886629	*KCNT2*	1	5.53E-06	3.6 [2.1-6.5]	
							rs7588295	*CSRNP3*	2	6.24E-06	8.6 [3.3-22.17]	
							rs4431170	*MARCH1*	4	7.28E-06	5.1 [2.5-10.6]	
							rs7740004	*C6orf170*	6	7.87E-06	5.9 [2.7-13.0]	
							rs11189381	*SFRP5*	10	8.17E-06	6.8 [2.9-15.8]	
							rs12903202	*ALDH1A2*	15	9.15E-06	4.0 [2.1-7.4]	
							rs17751934	*MEX3C*	18	9.16E-06	5.0 [2.4-10.1]	
							11–23990403	*LUZP2*	11	9.94E-06	12.7 [4.0-36.8]	

Table 6. Summary of candidate gene studies on CYP2C8 on ONJ

Year	Population	Underlying disease	BPs type	Case *n*	Control *n*	Genotyping	SNP	Gene	Chr	*p*-value	OR [95% CI]	Ref
2010	80% Caucasian 10% African American	Prostate cancer	Zoledronate Combination of BPs	17	83 BPs controls	Big Dye Terminator Cycle Sequencing Ready Reaction kit V3.1	rs1934951	*CYP2C8*	10	>0.47	0.63 [0.17-2.42]	(English et al., 2010)
2011	68% White 24% African American	Multiple myeloma	Zoledronate and/or Pamidronate	12	66 BPs controls	Taqman® Pyrosequencing	rs1934951 rs1934980	*CYP2C8* *CYP2C8*	10 10	0.63 0.66	0.68 [0.14-3.22] 0.70 [0.15-3.36]	(Katz et al., 2011)
2011	Caucasian	Multiple myeloma	Zoledronate	42	37 BPs controls 45 population controls	Taqman®	rs1934951	*CYP2C8*	10	0.13	/	(Such et al., 2011)
2012	Hungarian	Breast cancer Osteoporosis Multiple myeloma Prostate cancer	Zoledronate Ibandronate Pamidronate	46	224 population controls	Taqman®	rs1934951	*CYP2C8*	10	>0.05	/	(Balla et al., 2012)

Table 7. Summary of discovery candidate gene studies on ONJ

Year	Population	Underlying disease	BPs type	Case *n*	Control *n*	Genotyping	SNP	Gene	Chr	*p*-value	OR [95% CI]	Ref
2011	68% White 24% African American	Multiple myeloma	Zoledronate and/or Pamidronate	12	66 BPs controls	Taqman® Pyrosequencing	rs1800012 rs12458117 rs243865 rs2073618 rs3102735 rs11730582 rs28357094 rs1800629	*COL1A1* *RANK* *MMP2* *OPG* *OPG* *OPN* *OPN* *TNF*	17 18 16 8 8 4 4 6	0.55 0.38 0.11 0.38 0.75 0.21 0.41 0.67	1.69 [0.30-9.70] 2.14 [0.39-11.71] 3.49 [0.75-16.18] 2.16 [0.38-12.23] 0.79 [0.19-3.34] 2.97 [0.53-16.55] 0.51 [0.10-2.59] 0.68 [0.12-3.95]	(Katz et al., 2011)
2011	Italian	Breast cancer Multiple myeloma	Zoledronate	30	30 BPs controls 125 population controls	Taqman®	rs3025039 rs699947 rs2010963	 *VEGF* 	 6 	0.40 0.78 0.86	0.57 [0.21-1.54] 0.99 [0.31-3.18] 0.96 [0.37-2.53]	(Arduino et al., 2011)
2011	N/A	Multiple myeloma	Zoledronate	9	10 BPs controls	Affymetrix DMET™ plus platform 1,936 SNPs analysed	rs1152003 rs10893 rs4725373 rs1049793 rs2463437 rs903247 rs2468110 rs2097937	*PPARG* *ABP1* *CHST11* *CROT*	3 7 12 7	0.0055 0.023 0.023 0.023 0.0198 0.0198 0.0198 0.0198	/	(Di Martino et al., 2011)
2011	Caucasian	Breast cancer Multiple myeloma Prostate cancer	Zoledronate	34	34 BPs controls	GoTaq®	rs2297480	*FDPS*	1	0.03	/	(Marini et al., 2011)

Table 7 continued.

Year	Population	Underlying disease	BPs type	Case *n*	Control *n*	Genotyping	SNP	Gene	Chr	*p*-value	OR [95% CI]	Ref
2012	Caucasian	Breast cancer	Zoledronate	30	53 BPs controls	Taqman®	rs2234693	*ESR1*	6	>0.05	/	(La Ferla et al., 2012)
		Multiple myeloma					rs9340799	*ESR1*	6	>0.05	/	
		Prostate cancer					rs10046	*CYP19A1*	15	0.0439	2.83	
2013	White	Breast cancer	Zoledronate	94	110 BPs controls	LABType single strand oligonucleotide typing kit	DRB1*01	*MHC*	6	0.049	2.0 [0.99-4.1]	(Stockmann et al., 2013)
		Multiple myeloma	Pamidronate				DRB1*15			0.014	2.3 [1.2-4.4]	
			Combination of BP				DQB1*05:01			0.050	2.0 [0.99-4.0]	
		Prostate cancer					DQB1*06:02			0.014	2.3 [1.2-4.6]	

AIMS & OBJECTIVES

The aims of this study were to

- Validate ONJ cases and BPs-exposed controls in supporting the following analyses
- Identify potential clinical risk factors associated with BPs-associated ONJ development in a series of case-control analyses
- Identify potential genetic variants associated with BPs-associated ONJ development in a large, multicentre GWAS

The objective of case validation was to test whether the non-exposed ONJ cases are comparable to the exposed, so as to substantiate the inclusion of the non-exposed type in GENVABO analysis.

The objective of control validation was to test whether the controls had been adequately reviewed prior to recruitment to the GENVABO study.

As for clinical and genetic risk factor analyses, the objectives were to identify potential factors associated with the risk of ONJ development.

The STREGA (strengthening the reporting of genetic association studies) and STROBE (strengthening the reporting of observational studies in epidemiology) recommendations were followed in reporting the methods, results and discussion of the current study (von Elm et al., 2008; Little et al., 2009).

CHAPTER 2

Consortium Setting

Phenotyping and genotyping

2.1 Overview

The current study represents part of GENVABO, "GENetic VAriants as Biomarkers of jaw Osteonecrosis associated with bisphosphonates", a large, ongoing, international collaborative study led by the UCL/UCLH Eastman Dental Institute and Hospital.

The GENVABO Consortium was multidisciplinary and consisted of a clinical team and a genetic team. The clinical team was responsible for the following: application to the Ethical Committee, arrangement of collaboration agreement and "Material Transfer Agreement" with clinical centres, recruitment of study participants, collection of clinical data, blood and saliva samples, data entry, sample storage and management, as well as detailed clinical phenotyping and all related analyses.

The genetic team worked closely with the clinical team. It consisted of iSAEC, the international Serious Adverse Event Consortium, Dr Paola Nicoletti and Dr Yufeng Shen from the Columbia University Center for Computational Biology and Bioinformatics, and the University of Liverpool Wolfson Centre for Personalised Medicine. The team arranged DNA extraction from the biological samples, genotyping, and related association analyses.

2.2 Participant recruitment and clinical phenotyping

2.2.1 Participating clinical centres

This is a large, multicentre study with a total of 27 clinical centres from Europe and Asia (Table 8 and Table 9). In the discovery cohort, there were 23 centres, mostly from Italy, Spain and the UK. In this cohort, 393 BPs-associated ONJ

cases and 276 BPs-exposed controls were recruited. As for the replication cohort, 130 ONJ cases from seven European centres were recruited.

2.2.2 Participants inclusion criteria

Patients referred to the participating centres since January 2004 were eligible and the inclusion and exclusion criteria for both ONJ cases and BPs-exposed controls were detailed in Table 10.

The most updated ONJ definition at the beginning of the study, i.e. the AAOMS 2009 definition, which included the exposed type ONJ only, was adopted (Ruggiero et al., 2009). In addition, non-exposed cases were also recruited using the criteria suggested by Fedele et al., 2010. In fact, with the inclusion of the non-exposed, the GENVABO criteria was comparable to the most recent AAOMS 2014 definition (Ruggiero et al., 2014).

Of note, all participants had a head and neck examination performed by a clinician with experience and expertise in diseases of the mouth and jawbone. All ONJ cases were diagnosed and adjudicated by multidisciplinary teams of specialists in Oral Medicine, Oral and Maxillofacial Surgery, Oncology, Haematology, Rheumatology and Radiology.

2.2.3 Clinical phenotyping

Participants, including both cases and controls, required only a single visit for collection of clinical data and a blood or saliva sample for DNA extraction in the GWAS. The study was explained according to the participant information sheet (Appendix 8.1), and all participants gave informed consent (Appendix 8.2).

2.2.3.1 Case report form

A standardised Case Report Form (CRF) was used to gather clinical data for all study participants (Appendix 8.3). Information including demographics, primary underlying disease, BPs history, medical and dental history, were collected. These data were selected with reference to previous ONJ studies as discussed in Chapter 1 and were used for detailed analyses in Chapters 3, 4, 5 and 6.

As for ONJ cases, information regarding their ONJ features were also collected, including the site of the lesion, i.e. mandible and/or maxilla, ONJ type, i.e. exposed or non-exposed, dimension of the lesion, referring to the total length of exposed bone in millimetres, and lastly pain intensity, rated on a visual analogue scale (VAS 0-100mm) by the participant.

2.2.3.2 Data management

Data collection was performed between October 2008 and January 2015. The CRFs were stored in secured facilities at the UCL/UCLH Eastman Dental Institute and Hospital. Data were first transferred into electronic spreadsheets by two independent researchers via double entry process and were then reviewed by a central study panel, followed by data checking and verification.

2.2.3.3 Discovery GWAS cases

393 ONJ cases were recruited and their demographics, medical and dental history can be found in Table 11. Majority of the participants were female and the top three underlying diseases managed with BPs were osteoporosis, multiple myeloma and metastatic breast cancer. Therefore, over 80% were managed with zoledronate or alendronate. Regarding the prevalence of the potential risk factors amongst these cases, about 50% had history of

dentoalveolar surgery, i.e. tooth extraction and/or implant surgery, followed by use of steroids and smoking in about 20% of patients respectively.

Table 12 summarises ONJ features of the cases. About 60% of the lesions were in the mandible and 25% in the maxilla. ONJ was dominated by the exposed type and about 10% was the non-exposed. The two types were compared and will be reported in the next Chapter. The median of jawbone exposure was about 1cm, while the pain intensity median was about 2-3 out of 10.

As mentioned in Chapter 1, genetic variants are ethnicity-specific. Therefore, for the moment, only Caucasian cases were analysed and the 358 Caucasian cases, out of the overall cohort of 393 cases, were matched with 2,554 Caucasian population controls so as to achieve an approximately 1:10 case-control ratio in the discovery GWAS.

Population controls refer to individuals not exposed to BPs whose anonymous genotype data had been collected in previous studies and their database was made available for research purpose (Table 13). The GENVABO population controls data had been selected by the genetic team and were age-, gender- and ethnicity-matched with cases recruited by the clinical team.

2.2.3.4 Replication cases

As discussed in Chapter 1, the replication study is to confirm the discovery GWAS results and should be carried out in an independent cohort, which is ethnically and phenotypically similar to the discovery cohort. In GENVABO, in addition to the 393 cases in the discovery cohort, 130 new cases have been recruited since October 2013 for the ongoing replication study.

Ethnically, the current replication cases were all recruited in European clinical centres and 127 out of 130, i.e. 97.7%, were Caucasians (Table 8 and Table 9). Phenotypically, similar to the discovery case cohort, over 70% were female and about 70% were managed with zoledronate or alendronate, with similar BPs duration (Table 14). However, the replication cohort had a smaller proportion of multiple myeloma patients, and also fewer surgically-induced cases, as well as fewer exposed ONJ cases.

Further comparison between the discovery and the replication cohort cases was performed by the clinical team and will be reported in Chapter 6.

2.2.3.5 BPs-exposed controls

BPs-exposed controls were also recruited by the GENVABO clinical team during the same period when the 393 discovery cohort cases were recruited.

There were altogether 276 thoroughly phenotyped drug-exposed controls and their demographics, medical and dental history were reported in Table 11. They shared similar average age and gender proportion with the cases, the same top three underlying diseases managed with BPs, but different BPs history with regard to type and duration. Their comparison with the discovery cohort cases, so as to investigate the ONJ clinical risk factors, will be reported in Chapter 5.

Moreover, novel validation of these controls through comparing their follow-up time with the cases' time to ONJ onset was also carried out by the GENVABO clinical team and will be discussed in Chapter 4.

Table 8. List of participating clinical centres

Country	Centres in discovery GWAS, *n*	Cases in discovery GWAS, *n*	Drug-exposed controls, *n*	Centres in replication, *n*	Cases in replication, *n*
Italy	12	247	258	4	70
Spain	4	61	12	/	/
Hungary	/	/	/	1	50
United Kingdom	3	29	0	2	10
Japan	1	19	0	/	/
Sweden	1	17	1	/	/
Austria	1	13	4	/	/
China, Hong Kong	1	7	1	/	/
Total	**23**	**393**	**276**	**7**	**130**

Table 9. Full list of clinical centres

Discovery GWAS

Italy

1) Centro di Riferimento Oncologico della Basilicata, Istituto di Ricovero e Cura a Carattere Scientifico, Rionero in Vulture
2) Ospedale Civile di Alessandria, Alessandria
3) Sapienza University of Rome, Rome
4) Second University of Naples, Naples
5) University of Naples Federico II, Naples
6) University of Milan, Milan
7) University of Padua, Padua
8) University of Palermo, Palermo
9) University of Parma, Parma
10) University of Turin, Turin
11) University of Turin, Lingotto
12) University of Verona, Verona

Spain

1) Complexo Hospitalario Universitario de Coruña (CHUAC), La Coruña
2) Complexo Hospitalario Universitario de Santiago de Compostela (CHUS), A García
3) Policlínico Vigo S.A. (POVISA), Vigo
4) University of Valencia, Valencia

United Kingdom

1) Aintree University Hospital, Liverpool
2) UCL/UCLH Eastman Dental Institute and Hospital, London
3) University of Liverpool, Liverpool

Japan

Hyogo College of Medicine, Hyogo

Sweden

Uppsala University, Uppsala

Austria

Medical University of Graz, Graz

China, Hong Kong

University of Hong Kong

Replication study

Italy

1) Chirurgia Maxillo-Facciale, AOU Sassari
2) University of Palermo, Palermo
3) University of Parma, Parma
4) Ospedale S. Francesco, Nuoro

Hungary

Semmelweis University, Budapest

United Kingdom

1) King's College Hospital, London
2) UCL/UCLH Eastman Dental Institute and Hospital, London

Table 10. Inclusion and exclusion criteria

Inclusion criteria for all participants	
• Age over 18 • Capable of understanding the purpose of the trial and giving informed consent • On BPs medications	
Inclusion criteria for ONJ cases	**Inclusion criteria for BPs-exposed controls**
Exposed ONJ *AAOMS definition 2009* *(Ruggiero et al., 2009)* • Chronic non-healing exposure of one or more areas of the jawbones through the oral cavity and/or facial skin (longer than 8 weeks) • Chronic pain, infection, purulent discharge, abscess, fistulas **Non-exposed ONJ** *(Fedele et al., 2010)* • Unexplained jaw bone pain, fistula tract, bone or gingival swelling, not caused by dental or other bone disease	• No signs or symptoms of ONJ diagnosed on the basis of currently accepted criteria
Exclusion criteria for all participants	
• Age under 18 • Incapable of understanding the purpose of the trial and giving informed consent • Not on BPs medications	
Exclusion criteria for ONJ cases	**Exclusion criteria for BPs-exposed controls**
• History of radiation therapy to the head and neck region • No ONJ	/

Table 11. Discovery GWAS cases and BPs-exposed controls

		Cases ***N* = 393**		**Controls** ***N* = 276**	
		n	**%**	***n***	**%**
Age, *decade*	Mean, median	6.9, 7.0		6.6, 6.6	
	SD	0.9		1.1	
	Range	3.7 to 8.9		3.5 to 8.8	
Gender	Female	278	70.7%	168	60.9%
	Male	115	29.3%	101	36.6%
Primary underlying disease	Multiple myeloma	103	26.2%	107	38.8%
	Osteoporosis	137	34.9%	49	17.8%
	Metastatic breast cancer	89	22.6%	63	22.8%
	Metastatic prostate cancer	37	9.4%	38	13.8%
	Other cancers	27	6.9%	14	5.1%
BPs with longest duration	Zoledronate	230	58.5%	204	73.9%
	Alendronate	109	27.7%	31	11.2%
BPs duration, *year*	Mean, median	3.7, 2.8		2.6, 1.7	
	SD	3.1		2.8	
	Range	0.1 to 19.9		0.1 to 20.4	
Systemic factor	Diabetes	36	9.2%	19	6.9%
	Smoking	81	20.6%	47	17.0%
	Steroids	82	20.9%	42	15.2%
	Antiangiogenics	57	14.5%	94	34.1%
History of dentoalveolar surgery		196	49.9%	/	

Table 12. ONJ features of discovery GWAS cases

ONJ feature		*N* = 393	
		n	%
Site	Maxilla	97	24.7%
	Mandible	242	61.6%
	Both jaws	36	2.3%
Type	Exposed	344	87.5%
	Non-exposed	39	9.9%
Total dimension of jawbone exposure, *mm*	Mean, median	18, 10	
	SD	17	
	Range	0 to 105	
Pain intensity, *VAS 0-100mm*	Mean, median	32, 25	
	SD	33	
	Range	0 to 100	

Table 13. Population controls in discovery GWAS

Database	Ethnicity	*n*	Chip
POPRES	Caucasian	643	1M Illumina
HYPERGENE	Italian	901	1M Illumina
Penicillin Drug exposed CTLs	Italian	161	HumanOmniExpress BeadChip
TSI	Italian	99	1M Illumina
JAVIER-SP	Spanish	380	1M Illumina
Controls SPANISH	Spanish	200	1M Illumina
WTCCC	British	200	1M Illumina
SW CONTROLS	Swedish	250	1M Illumina

Table 14. Replication cases

		Cases *N* = 130	
		n	%
Age, *decade*	Mean, median	6.9, 7.0	
	SD	1.0	
	Range	4.3 to 8.8	
Gender	Female	99	76.2%
	Male	31	23.8%
Primary underlying disease	Metastatic breast cancer	43	33.1%
	Osteoporosis	42	32.3%
	Metastatic prostate cancer	18	13.8%
	Multiple myeloma	15	11.5%
	Other cancers	12	9.2%
BPs with longest duration	Zoledronate	66	50.8%
	Alendronate	27	20.8%
BPs duration, *year*	Mean, median	3.6, 2.8	
	SD	3.0	
	Range	0.2 to 15.1	
History of dentoalveolar surgery		42	32.3%
ONJ onset time, *year*	Mean, median	4.2, 3.2	
	SD	3.4	
	Range	0.2 to 15.9	
ONJ type	Exposed	101	77.7%
	Non-exposed	28	21.5%

2.3 Sample management and GWAS analysis

2.3.1 Biological samples

Blood samples from 393 discovery cohort cases, 122 replication cohort cases and 276 BPs-exposed controls were collected. Venepuncture using EDTA vacutainer tubes was performed to collect 6mL of blood.

Transfer of samples in dry ice from clinical centres to the UCL/UCLH Eastman Dental Institute and Hospital was done via a professional logistic company, BIOCAIR®. All samples were then stored at -80℃.

Eight replication cases donated saliva samples through a DNA collection kit, DNA Genotek®. Two millilitres of saliva was collected and was processed according to the kit's instruction. Processed samples were then stored at the Eastman Dental Institute and Hospital at -20℃.

All blood and saliva samples had been labelled with standardised coding.

2.3.2 DNA extraction and genotyping

Genomic DNA isolation was carried out amongst the 393 discovery cohort cases and 276 BPs-exposed controls. Recruitment of the replication cases is still in progress and their DNA extraction will be arranged in the near future.

DNA extraction for the cases was performed using the QIAamp® DNA Blood Mini Kit by Expression Analysis® in the United States, while DNA of the controls was extracted with the chemagen Magnetic Separation Module I, in the Wolfson Centre for Personalised Medicine, University of Liverpool, UK.

Extracted DNA quantity was assessed using the NanoDrop™ spectrophotometer, followed by normalisation to a fixed concentration. All samples were bar-coded and stored at -80℃.

At present, only the discovery GWAS cases have been genotyped, using high-throughput Illumina® Human1M Omni Express Beadchip. This platform contained 1,072,820 probes for SNPs and Copy Number Variations (CNVs) typing. Genotyping of the replication cases and the BPs-exposed controls is to be arranged.

2.3.3 GWAS analysis

As said, the 358 discovery GWAS cases were age-, gender- and ethnicity-matched with 2,554 population controls. Associations between genetic variants and ONJ were tested using logistic regression and Fisher's Exact test through PLINK, a statistical software for GWAS; performed by the genetic team. The main results will be presented in Chapter 6.

Figure 4. DNA extraction and genotyping

First row, left to right: EDTA vacutainer tube. Genotek® DNA collection kit. chemagen Magnetic Separation Module I automated genomic DNA extraction.

Second row. NanoDropTM spectrophotometer. Illumina® Human1M Omni Express.

CHAPTER 3

Case Cohort Validation

Exposed type versus non-exposed type ONJ

3.1 Introduction

3.1.1 Literature review

When ONJ was first reported, it was widely believed that its most characteristic feature was exposed necrotic jawbone, which has largely defined ONJ in the past decade (Sambrook et al., 2006; Khosla et al., 2007; Ruggiero et al., 2009).

Since 2008, non-exposed ONJ cases, who may represent up to one-third of all ONJ cases, has been increasingly reported (Junquera and Gallego, 2008; Mawardi et al., 2009; Woo et al., 2009; Fedele et al., 2010; Hutchinson et al., 2010; Truong et al., 2010; Kang et al., 2011b; Bagan et al., 2012; Patel et al., 2012; Wigler et al., 2013; Schiodt et al., 2014; Fedele et al., 2015). This was followed by numerous suggestions urging the inclusion of the non-exposed type into the definition and staging of ONJ (Colella et al., 2009; Mawardi et al., 2009; Yarom et al., 2010; Bedogni et al., 2012; Campisi et al., 2014). In 2014, “bone that can be probed through an intraoral or extraoral fistula(e)”, i.e. the non-exposed type, was finally included into the AAOMS definition (Ruggiero et al., 2014).

Therefore, for the first time, non-exposed type cases were considered for analysis in the current GWAS. This was further supported by early evidence indicating that the two types were similar, with regard to demographics, underlying diseases, medical history and clinical features (Schiodt et al., 2014; Fedele et al., 2015).

However, whether or not the same applies to the current case cohort remained unknown.

3.1.2 Objectives

The objective is to test the hypothesis that the non-exposed ONJ cases are comparable to the exposed type in the current cohort, so as to substantiate the inclusion of the non-exposed type in GENVABO analysis.

3.2 Methods

This part of the study involves secondary analysis of GENVABO clinical phenotype data.

3.2.1 Defining ONJ types

The dimension of necrotic bone exposure, correct to the nearest millimetre, was recorded for each case in the CRF.

The exposed type ONJ was defined as clinically evident necrotic jawbone, with a total dimension of bone exposure larger than 0.0 cm, which was visible through the oral mucosa or facial skin. For example, a case with 0.5 cm bone exposure was considered the exposed type. For individuals who were presented with more than one site of ONJ at the same time, the total dimension was calculated. For example, a case presenting with an ONJ site of 0.0 cm and another site of 3.0 cm would be categorised as the exposed type.

The non-exposed type had no frank bone exposure, i.e. 0.0 cm in total, but was still presented with clinical features including jawbone pain, sinus tract, bone enlargement, gingival swelling or any other signs, that were not caused by common jawbone diseases such as odontogenic infections, or other bone disorders with similar manifestations (Fedele et al., 2010; Patel et al., 2012).

3.2.2 Outcomes

3.2.2.1 Primary outcome

The primary aim of the present analysis was to compare the two types using descriptive statistics. The primary objective was to detect if there were any major numerical differences in their phenotype data.

3.2.2.2 Secondary outcome

The secondary aim was to compare the two types using inferential statistics. The secondary objective was to detect if there were any phenotypically, statistically significant differences between the exposed and non-exposed types.

3.2.3 Statistical analysis

Related data were transferred into electronic spreadsheets. All analyses were performed in Stata version 12.1 (Stata Corp., College Station, TX, US) and all graphs were constructed in Microsoft Excel 2013.

3.2.3.1 Primary outcome

Phenotypic features were reported using descriptive statistics. Mean, median, standard deviation and range were calculated for numerical data, including age, BPs duration and ONJ onset time. Numbers and percentages were calculated for categorical data, including gender, underlying disease, BPs type, systemic factors and history of dentoalveolar surgery. The percentages calculated were also plotted in a bar chart.

3.2.3.2 Secondary outcome

Each phenotypic feature formed the outcome variable, and ONJ type as the explanatory variable, exposed type=1 and non-exposed type=0.

The association between the explanatory variable and each numerical outcome variable, including age, BPs duration and ONJ onset time, was investigated with random-effects univariable linear regression. For binary outcome variables, including gender, underlying diseases, BPs type, systemic factors, and dentoalveolar surgery history, random-effects univariable logistic regression was used.

Multilevel random-effects were used to account for the clustering effect attributed to the participants being recruited in seven countries. The significance level for these analyses was 5%.

3.3 Results

3.3.1 Exposed type versus non-exposed type ONJ; descriptive statistics

344 participants (89.8%) had exposed ONJ, while 39 (10.2%) were of the non-exposed type (Table 15).

The two types had the same age median of 70 years. Both types had more female than male participants, although the non-exposed type had approximately 15% more females than the exposed type. They also shared the same top three underlying diseases: osteoporosis, multiple myeloma and metastatic breast cancer. Both had more participants on zoledronate than on alendronate, as well as similar BPs duration median of approximately three years, although the exposed type had nearly 15% more patients on zoledronate. They also shared very similar proportion of patients with history of smoking, and similar proportion of patients with history of dentoalveolar surgery, mainly tooth extraction and implant surgery.

However, there were more patients with diabetes and on steroids amongst the exposed type than the non-exposed, whereas there were more patients on antiangiogenics amongst the non-exposed cases. Lastly, ONJ onset time was longer amongst the non-exposed cases than the exposed.

The percentages calculated are plotted in a bar chart (Graph 1). The major differences were with use of steroids (20.1%), followed by gender (female) (15.4%), then with alendronate (15.1%), and zoledronate (14.3%). The rest differed by less than 10%.

3.3.2 Exposed type versus non-exposed type ONJ; inferential statistics

In total, 16 comparisons between the two types were performed and only three were found statistically significant (p<0.05) (Table 16).

With reference to the non-exposed cases, there was a statistically significantly larger proportion of exposed type cases who were managed with zoledronate (OR=2.10, 95% CI 1.05 to 4.19, p=0.036). In contrast, there was a statistically significantly smaller proportion of exposed type cases who were prescribed with alendronate (OR=0.44, 95% CI 0.22 to 0.89, p=0.023). On the other hand, the proportion of the exposed type cases who were on steroids was also statistically significantly larger than that of the non-exposed cases (OR=10.15, 95% CI 1.36 to 75.60, p=0.024).

Of note, the other 13 outcome variables, including age, gender, underlying diseases, BPs duration, three other systemic factors, history of dentoalveolar surgery, and ONJ onset time, were all found not statistically significant (p>0.05). Furthermore, the estimated coefficient for age was very close to zero, this implied that the two groups had very similar age (estimated coefficient 0.03 decades).

Table 15. Exposed type versus non-exposed type ONJ; descriptive statistics

		Exposed ONJ *N* = 344		Non-exposed ONJ *N* = 39	
		n	**%**	***n***	**%**
Age, *decade*	Mean, median	6.9, 7.0		6.9, 7.0	
	SD	1.0		0.8	
	Range	3.7 to 8.9		4.9 to 8.8	
Gender	Female	238	69.2%	33	84.6%
	Male	106	30.8%	6	15.4%
Primary underlying disease	Osteoporosis	117	34.0%	16	41.0%
	Multiple myeloma	92	26.7%	8	20.5%
	Metastatic breast cancer	75	21.8%	12	30.8%
	Metastatic prostate cancer	35	10.2%	2	5.1%
	Other cancers	25	7.3%	1	2.6%
BPs with longest duration	Zoledronate	208	60.5%	18	46.2%
	Alendronate	89	25.9%	16	41.0%
BPs duration, *year*	Mean, median	3.7, 2.8		4.1, 3.1	
	SD	3.1		3.3	
	Range	0.1 to 19.9		0.2 to 11.0	
Systemic factor	Diabetes	34	9.9%	1	2.6%
	Smoking (no=0, yes/ex=1)	71	20.6%	8	20.5%
	Steroids	78	22.7%	1	2.6%
	Antiangiogenics	47	13.7%	9	23.1%
History of dentoalveolar surgery		176	51.2%	17	43.6%
ONJ onset time, *year*	Mean, median	4.0, 3.1		4.5, 4.4	
	SD	3.2		3.3	
	Range	0.1 to 19.9		0.2 to 11.3	

Graph 1. Exposed type versus non-exposed type ONJ; differences in percentages

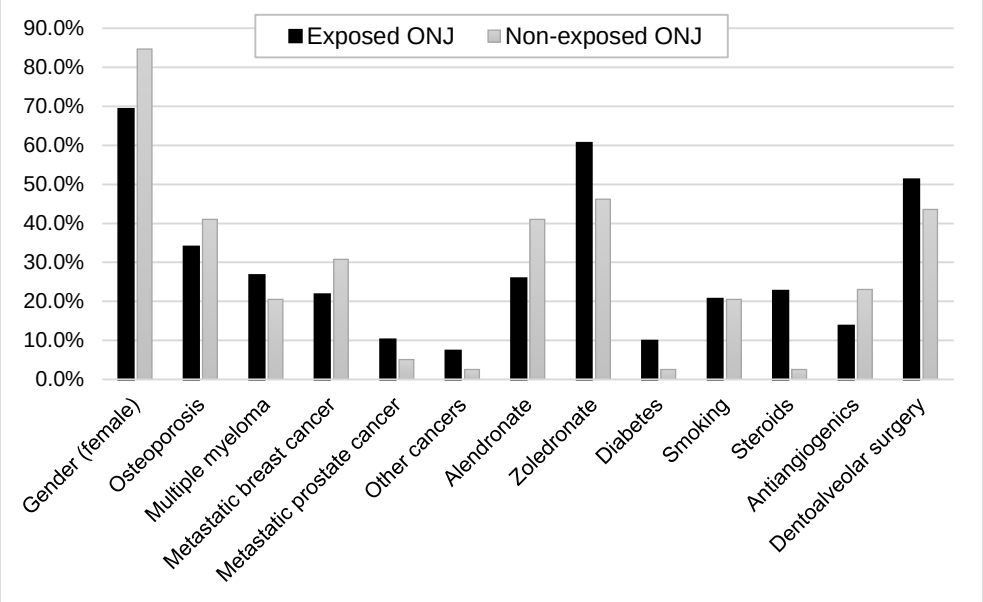


Table 16. Exposed type versus non-exposed type ONJ; random-effects univariable regression

Numerical outcome variable		*N* = 383; exposed ONJ=1, non-exposed ONJ=0		
		Estimated coefficient	**95% CI**	***p*-value**
Demographics	Age, *decade*	0.03	-0.28 to 0.34	0.868
BPs duration, *year*		-0.52	-1.56 to 0.51	0.319
ONJ onset time, *year*		-0.48	-1.55 to 0.58	0.373
Binary outcome variable		**OR**	**95% CI**	***p*-value**
Demographics	Gender (M=0, F=1)	0.41	0.17 to 1.00	0.051
Primary underlying disease	Osteoporosis	0.59	0.29 to 1.20	0.146
	Multiple myeloma	1.56	0.69 to 3.57	0.287
	Metastatic breast cancer	0.65	0.31 to 1.35	0.246
	Metastatic prostate cancer	2.10	0.48 to 9.07	0.322
	Other cancers	3.36	0.44 to 25.74	0.243
BPs with longest duration	Alendronate	0.44	0.22 to 0.89	0.023*
	Zoledronate	2.10	1.05 to 4.19	0.036*
Systemic factor	Diabetes	3.89	0.51 to 29.49	0.189
	Smoking (no=0, yes/ex=1)	0.92	0.40 to 2.16	0.854
	Steroids	10.15	1.36 to 75.60	0.024*
	Antiangiogenics	0.53	0.24 to 1.18	0.120
History of dentoalveolar surgery		1.42	0.72 to 2.81	0.310

* Significant results, $p<0.05$

```
Random-effects ML regression                    Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                LR chi2(1)         =      0.03
Log likelihood  =  -519.6932                    Prob > chi2        =    0.8677

------------------------------------------------------------------------------
   agedecade |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   .0264535   .1587981     0.17   0.868    -.2847851    .3376922
       _cons |        6.9   .1504961    45.85   0.000     6.605033    7.194967
-------------+----------------------------------------------------------------
    /sigma_u |   4.15e-23   .1392553                            0           .
    /sigma_e |   .9398479   .0339581                     .8755933    1.008818
         rho |   1.95e-45   1.31e-23                            0           1
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.00 Prob>=chibar2 = 1.000




Random-effects ML regression                    Number of obs      =       364
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      52.0
                                                               max =       241

                                                LR chi2(1)         =      0.99
Log likelihood  = -928.81894                    Prob > chi2        =    0.3193

------------------------------------------------------------------------------
durationyearplus1mo |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------
               neve |  -.5245767   .5262421    -1.00   0.319    -1.555992    .5068389
              _cons |   4.391983   .6190267     7.09   0.000     3.178713    5.605253
--------------------+---------------------------------------------------------
           /sigma_u |   .7374955   .5088548                      .1907436     2.85147
           /sigma_e |   3.074501   .1161882                     2.855006    3.310872
                rho |   .0544092   .0716544                     .0020085    .3706146
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.50 Prob>=chibar2 = 0.239
```

```
Random-effects ML regression                    Number of obs      =       351
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      50.1
                                                               max =       240

                                                LR chi2(1)         =      0.79
Log likelihood  = -904.58442                    Prob > chi2        =    0.3731

------------------------------------------------------------------------------
onsettimeyearplus1mo |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
---------------------+--------------------------------------------------------
               neve |  -.4830353   .5419334    -0.89   0.373    -1.545205    .5791347
              _cons |   4.604974    .577175     7.98   0.000     3.473732    5.736216
---------------------+--------------------------------------------------------
            /sigma_u |   .4790235          .                           .           .
            /sigma_e |   3.166039   .1203842                      2.938668    3.411002
                 rho |   .0223796          .                           .           .
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.16 Prob>=chibar2 = 0.345
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      3.81
Log likelihood  = -229.19938                    Prob > chi2        =    0.0509

------------------------------------------------------------------------------
        m0f1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   .4081942    .187323    -1.95   0.051     .1660523    1.003434
       _cons |   5.499634   2.440753     3.84   0.000     2.304455      13.125
-------------+----------------------------------------------------------------
    /lnsig2u |  -14.20371   68.22386                      -147.92    119.5126
-------------+----------------------------------------------------------------
     sigma_u |   .0008236   .0280938                     7.58e-33    8.95e+25
         rho |   2.06e-07   .0000141                     1.75e-65           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      2.11
Log likelihood  = -230.66219                    Prob > chi2        =    0.1463

------------------------------------------------------------------------------
          op |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   .5891809    .214569    -1.45   0.146     .2885736    1.202931
       _cons |   1.968203   1.084531     1.23   0.219      .668399    5.795677
-------------+----------------------------------------------------------------
    /lnsig2u |   -.009543   .7075365                     -1.396289    1.377203
-------------+----------------------------------------------------------------
     sigma_u |   .9952399   .3520843                      .4975076    1.990929
         rho |   .2314058   .1258404                      .0699709    .5464545
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    32.56 Prob >= chibar2 = 0.000
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      1.13
Log likelihood  = -217.56785                    Prob > chi2        =    0.2873

------------------------------------------------------------------------------
          mm |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   1.564167   .6576059     1.06   0.287     .6861544    3.565699
       _cons |    .124431   .0769226    -3.37   0.001     .0370442    .4179625
-------------+----------------------------------------------------------------
    /lnsig2u |  -.6448878   1.169962                     -2.937971    1.648195
-------------+----------------------------------------------------------------
     sigma_u |   .7243766   .4237465                      .2301589    2.279823
         rho |   .1375565   .1387981                      .0158467    .6123848
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     3.96 Prob >= chibar2 = 0.023
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      1.35
Log likelihood  = -204.36787                    Prob > chi2        =    0.2459

------------------------------------------------------------------------------
      mbcny0 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   .6468609   .2428396    -1.16   0.246     .3099269     1.35009
       _cons |   .3531666   .1812761    -2.03   0.043     .1291422    .9658082
-------------+----------------------------------------------------------------
    /lnsig2u |  -2.372953   2.484644                     -7.242766    2.496861
-------------+----------------------------------------------------------------
     sigma_u |   .3052952   .3792749                      .0267457    3.484869
         rho |   .0275504   .0665671                      .0002174    .7868453
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.19 Prob >= chibar2 = 0.330
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      0.98
Log likelihood  = -121.02974                    Prob > chi2        =    0.3224

------------------------------------------------------------------------------
         mpc |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   2.095471   1.566474     0.99   0.322     .4841322    9.069835
       _cons |   .0540541   .0392414    -4.02   0.000     .0130282    .2242704
-------------+----------------------------------------------------------------
    /lnsig2u |   -17.4872   1693.102                     -3335.907    3300.933
-------------+----------------------------------------------------------------
     sigma_u |   .0001595   .1350066                             0           .
         rho |   7.73e-09   .0000131                             0           .
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      1.36
Log likelihood  = -93.352532                    Prob > chi2        =    0.2434

------------------------------------------------------------------------------
 othercancer |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   3.359484       3.49     1.17   0.243     .4385325    25.73614
       _cons |    .015006   .0170489    -3.70   0.000     .0016187    .1391073
-------------+----------------------------------------------------------------
    /lnsig2u |  -1.016579   1.462125                     -3.882292    1.849134
-------------+----------------------------------------------------------------
     sigma_u |   .6015237   .4397515                      .1435394    2.520777
         rho |   .0990856   .1305205                      .0062238    .6588754
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     1.82 Prob >= chibar2 = 0.089
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      5.16
Log likelihood  = -218.23276                    Prob > chi2        =    0.0231

------------------------------------------------------------------------------
         ale |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   .4395922   .1590043    -2.27   0.023     .2163531    .8931757
       _cons |   1.231296   .5500004     0.47   0.641     .5130314    2.955158
-------------+----------------------------------------------------------------
    /lnsig2u |  -1.042213    .848733                     -2.705699    .6212734
-------------+----------------------------------------------------------------
     sigma_u |   .5938631   .2520156                      .2585026    1.364293
         rho |   .0968207   .0742187                      .0199076    .3613351
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     9.68 Prob >= chibar2 = 0.001
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      4.41
Log likelihood  = -247.69333                    Prob > chi2        =    0.0357

------------------------------------------------------------------------------
         zol |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   2.098226     .74042     2.10   0.036     1.050706    4.190091
       _cons |   .3744695    .186865    -1.97   0.049     .1408173    .9958106
-------------+----------------------------------------------------------------
    /lnsig2u |  -.4144077   .7559657                     -1.896073    1.067258
-------------+----------------------------------------------------------------
     sigma_u |   .8128539   .3072449                      .3875011    1.705109
         rho |   .1672484   .1052882                        .04365    .4691419
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    20.15 Prob >= chibar2 = 0.000
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      1.73
Log likelihood  = -115.15112                    Prob > chi2        =    0.1887

------------------------------------------------------------------------------
          dm |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       neve |   3.890536   4.020908     1.31   0.189      .513197    29.49408
       _cons |   .0269028   .0284034    -3.42   0.001     .0033972    .2130478
-------------+----------------------------------------------------------------
    /lnsig2u |  -1.502478    1.83603                     -5.101031    2.096074
-------------+----------------------------------------------------------------
     sigma_u |   .4717815   .4331025                      .0780414    2.852048
         rho |   .0633683   .1089735                      .0018479    .7120224
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.89 Prob >= chibar2 = 0.172
```

```
Random-effects logistic regression              Number of obs      =       323
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      46.1
                                                               max =       186

                                                Wald chi2(1)       =      0.03
Log likelihood  = -179.66959                    Prob > chi2        =    0.8544

------------------------------------------------------------------------------
     smoking |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       neve |   .9236843   .3995445    -0.18   0.854     .3956688     2.15633
       _cons |   .3477618   .1427491    -2.57   0.010     .1555539    .7774684
-------------+----------------------------------------------------------------
    /lnsig2u |  -14.70182   69.34299                     -150.6116     121.208
-------------+----------------------------------------------------------------
     sigma_u |    .000642   .0222594                     1.97e-33    2.09e+26
         rho |   1.25e-07   8.69e-06                     1.18e-66           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      5.12
Log likelihood  =  -187.7025                    Prob > chi2        =    0.0237

------------------------------------------------------------------------------
     steroid |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       neve |   10.15164   10.39977     2.26   0.024     1.363102    75.60381
       _cons |   .0338146    .035019    -3.27   0.001     .0044421    .2574088
-------------+----------------------------------------------------------------
    /lnsig2u |  -2.191413   1.254914                     -4.650999    .2681736
-------------+----------------------------------------------------------------
     sigma_u |   .3343034    .209761                      .0977346    1.143492
         rho |   .0328545    .039875                      .0028951    .2844134
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     2.19 Prob >= chibar2 = 0.069
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      2.42
Log likelihood  = -158.25332                    Prob > chi2        =    0.1199

------------------------------------------------------------------------------
     antiang |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        neve |   .5275138   .2169249    -1.56   0.120     .2356143    1.181044
       _cons |   .2999513   .1140041    -3.17   0.002     .1424065    .6317883
-------------+----------------------------------------------------------------
    /lnsig2u |  -14.34109   68.47682                     -148.5532     119.871
-------------+----------------------------------------------------------------
     sigma_u |   .0007689    .026326                      5.52e-33    1.07e+26
         rho |   1.80e-07   .0000123                      9.27e-66           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       383
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      54.7
                                                               max =       243

                                                Wald chi2(1)       =      1.03
Log likelihood  = -262.64982                    Prob > chi2        =    0.3102

------------------------------------------------------------------------------
alveolarsurgery |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
----------------+-------------------------------------------------------------
           neve |   1.421923   .4932651     1.01   0.310     .7204344    2.806454
          _cons |   .5874569    .279619    -1.12   0.264     .2311103    1.493251
----------------+-------------------------------------------------------------
       /lnsig2u |   -.604392   1.015532                     -2.594799    1.386015
----------------+-------------------------------------------------------------
        sigma_u |   .7391932   .3753373                      .2732415     1.99972
            rho |   .1424316    .124042                      .0221906    .5486375
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     4.82 Prob >= chibar2 = 0.014
```

3.4 Discussion

3.4.1 Main findings and comparison with previous studies

Current results were mostly similar to previous studies, which further confirmed that non-exposed cases were largely comparable to the exposed type counterparts.

For demographics, the two types shared nearly the same mean age and median. As for gender, both had more female than male participants and the difference in proportions was not statistically significant. These were all consistent with Schiodt et al., 2014 and Fedele et al., 2015.

As for underlying diseases, similar to Schiodt et al., 2014, there was no statistically significant difference in the proportion of patients presented with various diseases between the two groups. As for alendronate and zoledronate therapy, same as Fedele et al., 2015, the proportion of patients on different BPs types was found statistically significantly different between exposed and non-exposed type ONJ. On the other hand, BPs duration was found similar.

Results on diabetes and smoking history were also similar to Schiodt et al., 2014, in which there were no major differences between the two groups. In the current cohort, there were significantly more exposed ONJ cases on steroids, but the proportion of patients on steroids was similar in the two groups in Fedele et al., 2015. The use of antiangiogenics has not been studied previously and was found similar between the two types in the current study.

The current study and Fedele et al., 2015 found more exposed type cases reporting history of dentoalveolar surgery, whereas Schiodt et al., 2014 found

more non-exposed cases which were surgically-triggered. Of note, only Fedele et al., 2015 calculated a significant difference between the two ONJ types in the proportion of patients having tooth extractions before ONJ development. ONJ onset time has not been studied previously and the current study found that ONJ manifested about six months earlier in the exposed cases than in the non-exposed cases, but the difference was not statistically significant.

Therefore, it can be summarised that the current analysis shared similar results with previous studies and that the non-exposed ONJ was found largely comparable to the exposed type. However, evidence supporting the two types as similar was based on results from three early studies only. Moreover, apart from the statistically significant differences between the two regarding use of alendronate, zoledronate, and steroids, there could still be notable differences although not statistically significant. For instance, there were 9.4% more non-exposed cases who were managed with antiangiogenics, while p=0.120. Similar to patients diagnosed with metastatic breast cancer, the difference in proportion was 9.0%, while p=0.246. Also, there remained aspects that have not been tested but could be different between the two types.

Nevertheless, all the available studies, i.e. Schiodt et al., 2014, Fedele et al., 2015 and the current study demonstrated that exposed and non-exposed ONJ are largely similar, with regard to demographics, underlying diseases, medical history, as well as ONJ onset event and time. There was also evidence suggesting that the two types are radiologically similar (Mawardi et al., 2009; Hutchinson et al., 2010).

Further supported by the recent inclusion of the non-exposed type into the AAOMS definition (Ruggiero et al., 2014), therefore, for the first time, these cases would be included into an ONJ GWAS, i.e. GENVABO, and would be analysed together with the exposed counterparts.

3.4.2 Study strengths

3.4.2.1 Current study comparable to previous studies

The study design of the current study was similar to Schiodt et al., 2014 and Fedele et al., 2015, in particular their statistical analyses, which enabled direct comparison of their results.

Of note, there may be an overlap of cases of the current study with Fedele et al., 2015 as some of the clinical centres participated in both studies. Yet, the number and ratio of exposed and non-exposed cases were totally different. Therefore, the two studies remained different and should be considered as two independent studies.

3.4.2.2 Clear definition of non-exposed type ONJ

Another strength of the current analysis was the adoption of the definition of the non-exposed ONJ, described by Fedele et al., 2010 and Patel et al., 2012.

The current study classified each case when the individual was enrolled for the GENVABO study. However, as this definition does not specify the time point at which non-exposed features are observed, it may have included healing ONJ, which may be of the exposed type at an earlier time. On the other hand, it may have missed an initially non-exposed ONJ, which may present with exposed jawbone later. Nonetheless, this is currently the best available gold standard, although further revision may be necessary.

3.4.2.3 Robust statistical analysis

Detailed information was collected and thorough clinical phenotyping had been performed. New variables including the use of antiangiogenics and ONJ onset time were analysed for the first time. A bar chart was also plotted to illustrate the difference in percentages for each variable between the two types. In the univariable regressions, multilevel random-effects were used to account for the clustering effect attributed to the participants being recruited in seven countries. For the results, both descriptive statistics, i.e. mean, median, numbers and percentages, as well as inferential statistics, i.e. results from the univariable regressions, were carefully analysed.

3.4.3 Study limitations

Study limitations included missing data, relatively few non-exposed type cases and related issues.

Data on ONJ dimension were missing in 10 cases (2.5%), making their ONJ type unavailable and their analysis impossible.

In the current study, there were only 39 non-exposed cases versus 344 exposed ONJ, giving a ratio of approximately 1:8.8. In contrast, Fedele et al., 2015 recruited more non-exposed cases (*N*=192) and had a more favourable ratio of 1:3.2 versus the exposed. Schiodt et al., 2014 also had a more favourable ratio of the non-exposed versus the exposed (1:6.3). However, only 14 non-exposed cases were recruited.

As the overall cohort size of the non-exposed type was small, the numbers of non-exposed cases associated with different types of BPs were even smaller (zoledronate: *N*=18; alendronate: *N*=16). Therefore, due to statistical

consideration, cohort stratification according to BPs type as in Chapters 4, 5 and 6 had not been performed. In fact, to date, cohort stratification has never been performed in any of the previous studies (Schiodt et al., 2014; Fedele et al., 2015). However, both the current analysis and Fedele et al., 2015 found that different BPs types were significantly associated with different ONJ types. Therefore, in future, if cohort size allows, stratification may be attempted.

Similarly, there were even fewer non-exposed cases presented with certain medical history. Amongst the non-exposed cases, there was only one other cancer patient, one presented with diabetes and one managed with steroids. So, for these variables, they had a much wider 95% confidence interval for the OR, as well as a relatively larger standard error in the univariable regressions.

Therefore, although it was found that steroid users had a higher odds of having the exposed type, i.e. a statistically significant difference between the two types with regard to steroids use, there was only one non-exposed case who was on steroids, compared to 78 out of 344 exposed cases. As a result, its 95% confidence interval was wide (1.36 to 75.60), accompanied by a large standard error (10.40). In contrast, Fedele et al., 2015 identified 49 out of 192 non-exposed ONJ steroid users, and found no statistically significant difference between the two types. All in all, whether or not there was any difference between the two types with regard to the use of steroids remains controversial and inconclusive. Nonetheless, the non-exposed type is still considered comparable to the mainstream exposed ONJ.

3.5 Links to Chapters 5 and 6

As the two types were found largely phenotypically similar to each other in the current cohort, the non-exposed type cases will be analysed together with the exposed counterparts in the GWAS in Chapter 6.

To further show that they are similar, there will be additional risk factor analysis in Chapter 5. Results from cohorts with the exposed type cases only and with both exposed and non-exposed cases will be compared.

CHAPTER 4

Control Cohort Validation

Follow-up time versus time to onset

4.1 Introduction

4.1.1 Literature review

One essential element of a case-control study, including a GWAS, is the selection of controls, which basically refers to the identification of individuals who do not have the condition under investigation (Wacholder and Rotunno, 2009).

ONJ is known as a type C chronic or continuous, dose and time related ADR (Edwards and Aronson, 2000). However, there were studies which matched cases and controls with regard to age, gender or underlying diseases only, while BPs dose and length had been completely neglected (Kyrgidis et al., 2008; Wessel et al., 2008; Thumbigere-Math et al., 2013; Tsao et al., 2013).

There were studies which considered BPs dose and duration in matching cases and controls. Some included controls based on a minimum requirement of receiving one dose (Thumbigere-Math et al., 2012), three months (Sedghizadeh et al., 2013) or five months of BPs (Vahtsevanos et al., 2009). In contrast, a median BPs duration of 21.5 months amongst the zoledronate patients, and 42.0 months for pamidronate, were reported in a recent study of 963 ONJ patients (Gabbert et al., 2015).

Therefore, with the absence of, or low requirement in, BPs dose or duration screening, the controls in these studies may still present with a risk of having ONJ after being recruited, i.e. risk of being “false-controls”.

Another issue with controls selection through BPs duration screening is that there can be a time lag between end of BPs therapy and ONJ onset.

A recent study reported that ONJ can occur up to 10 years after BPs withdrawal (Parretta et al., 2014). Therefore, regardless of the length of BPs duration, if the controls had not be adequately reviewed, there may still be a risk of “false-control”.

To date, controls selection through follow-up time screening remains uncommon and experimental.

4.1.2 Objectives

The objective is to test whether the controls in the current cohort had been adequately reviewed prior to recruitment to the GENVABO study, through comparing the controls’ follow-up time with cases’ time to onset. The other objective is to develop a new method in selecting super-controls, i.e. those who had been more adequately reviewed and thus carry a lower risk of being “false-controls”.

4.2 Methods

This part of the study involves secondary analysis of GENVABO clinical phenotype data. It consists of three sets of follow-up time and time to onset comparisons, in the overall, zoledronate and alendronate cohorts, followed by super-controls selection for further analyses.

4.2.1 Defining time to onset and follow-up time

As described, a series of important time events were recorded in the CRF and used in calculating the following time periods.

“Time to onset” applies to ONJ cases and is defined as the number of years elapsed between the initiation of BPs therapy and ONJ diagnosis, i.e. when ONJ was diagnosed according to the diagnostic criteria mentioned in 2.2.2, adjudicated by a local multidisciplinary team of clinical specialists.

“Follow-up time” applies to BPs controls and is defined as time elapsed, in number of years, between initiation of BPs therapy and enrolment for the GENVABO study, i.e. the latest follow-up.

Of note, for both cases and controls, the initiation of BPs therapy refers to the time when patients received the first dose of BPs.

4.2.2 Time to onset versus follow-up time

4.2.2.1 Outcomes

4.2.2.1.1 Primary outcome

The primary aim of the present analysis was to compare cases’ time to onset with controls’ follow-up time, using descriptive statistics. The primary objective was to detect if there were any major numerical differences between onset time and follow-up time.

4.2.2.1.2 Secondary outcome

The secondary aim was to compare cases’ time to onset with controls’ follow-up time, using inferential statistics. The secondary objective was to detect if there were any statistically significant differences between onset time and follow-up time.

4.2.2.2 Statistical analysis

All analyses were performed in Stata version 12.1 (Stata Corp., College Station, TX, US) and all graphs were constructed in Microsoft Excel 2013.

4.2.2.2.1 Primary outcome

Time to onset and follow-up time were reported using descriptive statistics. Mean, median, standard deviation and range were calculated. The number and cumulative percentage of (i) cases being diagnosed at different lengths of time, and (ii) controls being reviewed at different lengths of time, were also calculated and plotted in various line charts.

4.2.2.2.2 Secondary outcome

Onset time or follow-up time formed the outcome variable, and ONJ development was the explanatory variable, case=0 and control=1.

The association between the explanatory variable and the outcome variable was investigated with random-effects univariable linear regressions.

Multilevel random-effects were used to account for the clustering effect attributed to the participants being recruited in seven countries. The significance level for these analyses was 5%.

4.2.3 Super-controls selection

Findings from the onset time and follow-up time comparisons were carefully considered. Different selection outcomes include (i) rejecting the entire control cohort, (ii) accepting the entire control cohort, or (iii) accepting part of the cohort who were super-controls, i.e. those who had been more adequately reviewed.

4.3 Results

Amongst the overall 393 cases, time events-related data were complete in 357 cases, including 212 zoledronate patients and 94 alendronate patients. As for controls, 272 out of 276 had complete time events data, in which 203 were zoledronate patients, and only 30 were on alendronate.

4.3.1 Time to onset versus follow-up time

4.3.1.1 Overall cohort

Considering all the cases and controls in the overall cohort, the controls' follow-up time ranged from 0.1 to 20.4 years and its median was 2.2 years, which was about a year shorter than the cases' time to onset (Table 17). The median onset time was 3.2 years and it ranged from 0.1 to 19.9 years.

Graph 2 presents the number of (i) cases being diagnosed, and (ii) controls being reviewed at different lengths of time. For instance, in the first year, ONJ was diagnosed in 49 individuals. In other words, these 49 cases had an onset time of within a year. There were also 63 controls who had been reviewed for within a year at recruitment to the GENVABO study.

In general, the numbers for both cases and controls were high in the first three years (*N*~50). For the cases, two peaks were observed in the second and in the fifth years. Only one peak was observed for the controls and it was also in the second year. In the first year, there were more controls recruited than cases being diagnosed. Whereas in the third, fifth to ninth, 11^{th}, 12^{th} and 15^{th} years, the cases outnumbered the controls.

Graph 3 presents the cumulative percentage of (i) cases being diagnosed, and (ii) controls being reviewed at different lengths of time. For instance, ONJ was diagnosed in 49 individuals in the first year and in another 65 cases in the second year. Therefore, the cumulative percentage of cases being diagnosed in the second year was calculated as (49+65)/357=31.9%. Similarly, the cumulative percentage of controls being reviewed in the second year was calculated as (63+65)/272=47.1%.

Both curves are of convex shapes. As there were more controls in the earlier years but more cases in later years, the control curve is on the left hand side of the case curve. From the first to the 11th year, the percentages amongst the controls were all higher than that of the cases. Afterwards, there was a smaller proportion of controls than cases.

It took about six to seven years to capture the vast majority of cases (~80%). However, the vast majority of controls (80.5%) had only been reviewed for five years or less. Only about 10% of controls had been reviewed for eight or more years.

Furthermore, it was found that the overall follow-up time was also statistically significantly shorter than the overall time to onset (estimated coefficient -0.82 years, 95% CI -1.34 to -0.31 years, p=0.002) (Table 20).

4.3.1.2 Zoledronate cohort

Similar to the overall cohort, the zoledronate controls' follow-up time was also found shorter than the cases' time to onset (Table 18). Their medians differed by 0.4 years (follow-up time: 1.7 years; onset time: 2.1 years).

Graph 4 compares onset time with follow-up time in terms of number of individuals. The case (*N*=212) and the control cohorts (*N*=203) were of similar sizes. At most time points, the number of controls being reviewed were found comparable to the number of cases. Both peaked in the second year, which corresponded with the peaks observed in the overall cohort. However, in the first year, there were 17 more controls being recruited than the cases. Whereas in the fifth and sixth years, there were 19 more cases than controls.

Graph 5. Zoledronate cohort onset time versus follow-up time; cumulative percentage over time

5 compares onset time with follow-up time with regard to cumulative percentage. The control curve is again on the left hand side of the case curve as there were more controls recruited in earlier years than in later years. From the first to the fourth years, there were more controls than cases, by approximately 10%. The difference in percentages then decreased and almost disappeared from the sixth year onwards.

It took about four to five years to capture the vast majority of cases (~80%). However, 75.4% of controls had only been reviewed for three years or less. Only about 10% of controls had been reviewed for six or more years.

Lastly, it was also found that the zoledronate controls' follow-up time was statistically significantly shorter than the cases' onset time (estimated coefficient -0.48 years, 95% CI -0.91 to -0.05 years, *p*=0.03) (Table 20).

4.3.1.3 Alendronate cohort

The alendronate controls' follow-up time was also found shorter than the cases' time to onset (Table 19). Their medians differed by 1.8 years (follow-up time: 4.2 years; onset time: 6.0 years).

Graph 6 compares the onset time with follow-up time amongst alendronate cases and controls in terms of number of individuals. In total, there were three times as many cases (*N*=94) as controls (*N*=30). In the first four years, the numbers were similar; in total, 16 cases and 14 controls were recruited respectively. The number of alendronate cases then peaked in the fifth year (*N*=18), which corresponded with the second peak observed amongst the overall cases in Graph 2. However, in the same year, only four controls were recruited and the numbers continued to decrease from the sixth year onwards.

The control curve is also on the left hand side of the case curve and their differences in cumulative percentages were also large (Graph 7).

It took about nine to 10 years to capture the vast majority of cases (~80%). However, 80.0% of controls had only been reviewed for eight years or less. Only about 10% of controls had been reviewed for 11 or more years.

Although alendronate controls' follow-up time was shorter than the cases' time to onset, the difference was not found statistically significant (estimated coefficient -1.05 years, 95% CI -2.41 to 0.31 years, *p*=0.129) (Table 20).

4.3.1.4 Summary

Controls' follow-up time, in terms of mean and median, were found shorter than cases' time to onset in all three cohorts: the overall, zoledronate and alendronate cohorts. Regression analyses also found that the follow-up time was statistically significantly shorter than the onset time in the overall and the zoledronate cohorts ($p<0.05$), but not in the alendronate cohort.

Both time to onset and follow-up time, in terms of mean, median and peak time of ONJ diagnosis, were found earlier amongst the zoledronate patients than those managed with alendronate.

There were similar number of cases and controls in the zoledronate cohort, and the overlap between the two curves is fairly good, except mainly in the first year when there were more controls than cases, right before the peak of ONJ diagnosis in the second year.

In the alendronate cohort, there were two times more cases than controls and the overlap between the two curves at most time points is poor.

All three cumulative percentage graphs show the control curve on the left hand side of the case curve as there were more controls recruited in earlier years than in later years.

4.3.2 Super-controls selection

As the zoledronate and the alendronate cohorts showed different time to onset, follow-up time, and case to control ratio, separate, dedicated super-control selections were performed.

4.3.2.1 Zoledronate cohort

In the zoledronate cohort, the controls' follow-up time was found statistically significantly shorter than the cases' time to onset. Therefore, the option of rejecting the entire zoledronate control cohort (*N*=203) was considered.

However, when each time point was considered, follow-up time was found comparable to the onset time. First, both numbers of cases (*N*=56) and controls (*N*=58) peaked in the second year. Second, the number of controls being reviewed were found mostly similar to the number of cases at most time points (Graph 4), except in the first year when there were 17 more controls than cases.

A follow-up time of within a year was not long enough to cover the peak of ONJ diagnosis in the second year. Therefore, these controls (*N*=57) may still present with the risk of having ONJ after recruitment into the study, i.e. risk of being "false-control", and are not ideal for case-control risk factor analysis.

Hence, having considered the peak time of diagnosis and the median onset time, both around the second year, the more precise and stringent median of 2.1 years was chosen as the threshold in classifying the super-controls. Eighty eight controls had a follow-up time of 2.1 years or longer and were therefore selected as super-controls for additional analysis in the next Chapter.

4.3.2.2 Alendronate cohort

Although the difference between the alendronate controls' follow-up time and cases' time to onset was not statistically significant, the decision of accepting the entire alendronate cohort (*N*=30) was considered inappropriate.

Amongst the alendronate cases, peak time of ONJ diagnosis was in the fifth year while the median onset time was 6.0 years. However, 60.0% of the alendronate controls had a follow-up time of five years or less.

If the same criteria for the zoledronate controls were adopted, i.e. the more stringent median to be chosen as the cut-off, only eight individuals had a follow-up time of 6.0 years or longer and qualified as super-controls in the alendronate cohort. Since this is too few for comparison with 94 cases, additional analysis will not follow due to statistical consideration.

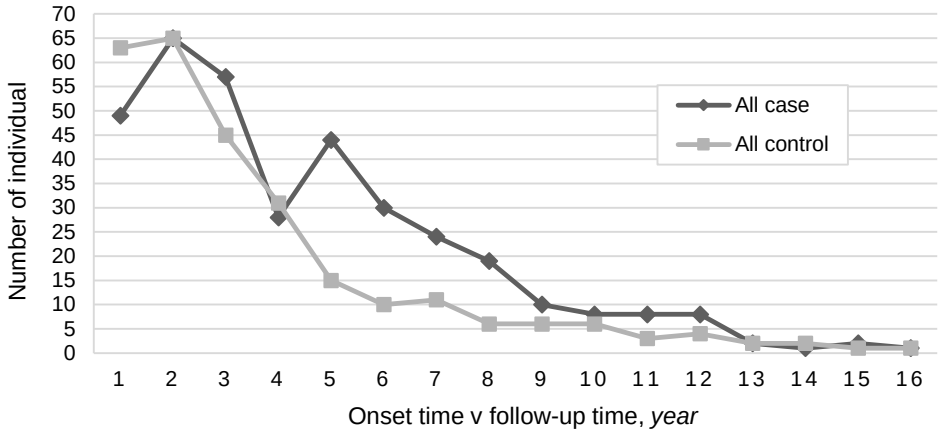
4.3.2.3 Summary

Findings from the onset time and follow-up time comparisons had been carefully considered. In the zoledronate cohort, the median onset time, 2.1 years, which was also around the peak time of diagnosis in the second year, was chosen as the cut-off in selecting super-controls. Eighty eight super-controls had a follow-up time of 2.1 years or longer and will be subject to additional risk factor analysis in Chapter 5. When the same approach was applied to the alendronate cohort, i.e. using the median of 6.0 years as cut-off, which was also around the peak time of diagnosis in the fifth year, only eight super-controls would be selected. As the cohort size it too small, there will not be additional analysis in Chapter 5.

Table 17. Overall cohort onset time versus follow-up time

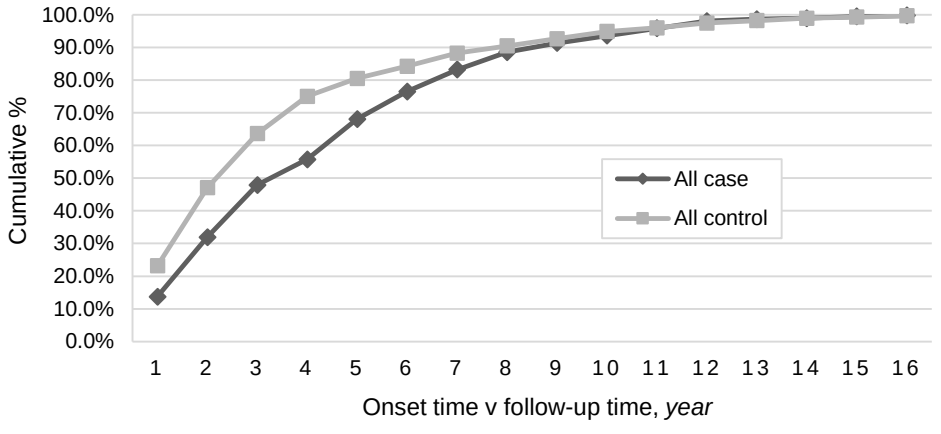
		Cases' onset time *N* = 357	Controls' follow-up time *N* = 272
Time, *year*	Mean; median SD; range	4.1; 3.2 3.2; 0.1 to 19.9	3.2; 2.2 3.2; 0.1 to 20.4

Graph 2. Overall cohort onset time versus follow-up time; number of individuals over time



Year, n^{th}	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
All case, *n*	49	65	57	28	44	30	24	19	10	8	8	8	2	1	2	1
All control, *n*	63	65	45	31	15	10	11	6	6	6	3	4	2	2	1	1

Graph 3. Overall cohort onset time versus follow-up time; cumulative percentage over time

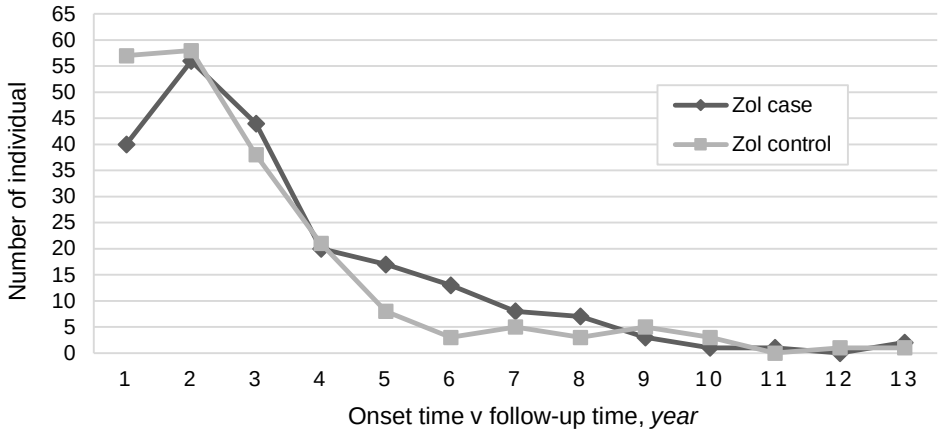


Year, n^{th}	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
All case, *%*	13.7	31.9	47.9	55.7	68.1	76.5	83.2	88.5	91.3	93.6	95.8	98.0	98.6	98.9	99.4	99.7
All control, *%*	23.2	47.1	63.6	75.0	80.5	84.2	88.2	90.4	92.6	94.9	96.0	97.4	98.2	98.9	99.3	99.6

Table 18. Zoledronate cohort onset time versus follow-up time

		Cases' onset time *N* = 212	Controls' follow-up time *N* = 203
Time, *year*	Mean; median SD; range	2.9; 2.1 2.3; 0.1 to 12.1	2.4; 1.7 2.2; 0.1 to 12.5

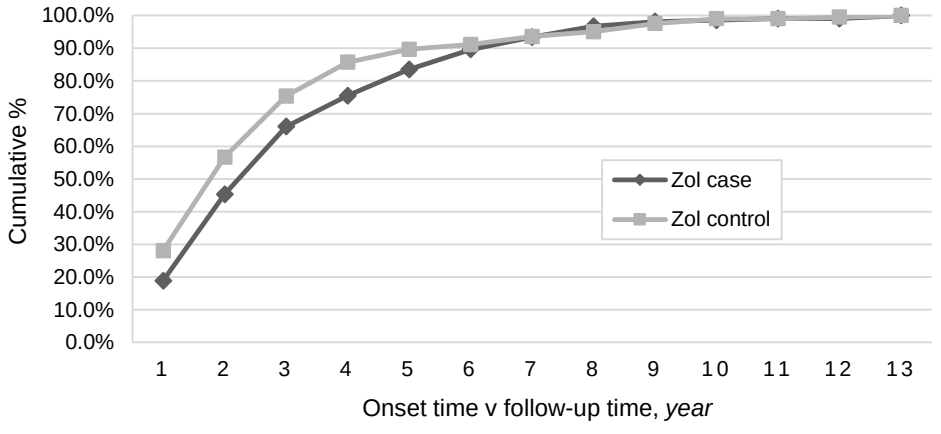
Graph 4. Zoledronate cohort onset time versus follow-up time; number of individuals over time



Year, n^{th}	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Zol case, *n*	40	56	44	20	17	13	8	7	3	1	1	0	2
Zol control, *n*	57	58	38	21	8	3	5	3	5	3	0	1	1

Zol = Zoledronate

Graph 5. Zoledronate cohort onset time versus follow-up time; cumulative percentage over time



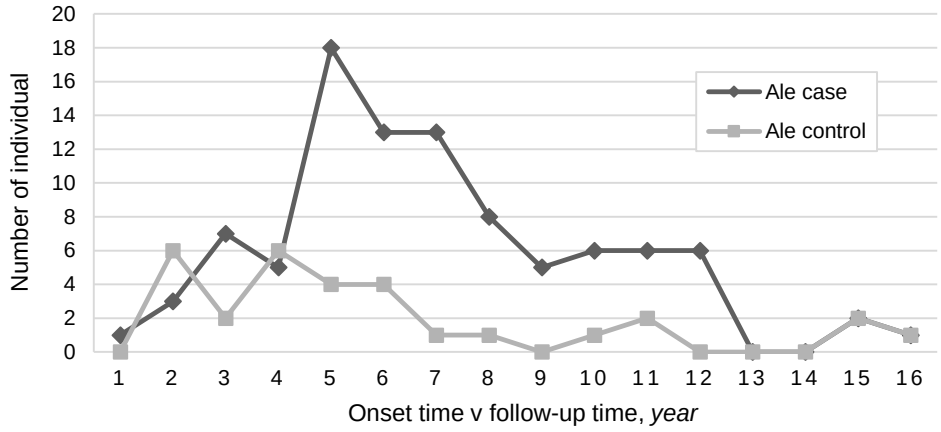
Year, n^{th}	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Zol case, *%*	18.9	45.3	66.0	75.5	83.5	89.6	93.4	96.7	98.1	98.6	99.1	99.1	100.0
Zol control, *%*	28.1	56.7	75.4	85.7	89.7	91.1	93.6	95.1	97.5	99.0	99.0	99.5	100.0

Zol = Zoledronate

Table 19. Alendronate cohort onset time versus follow-up time

		Cases' onset time *N* = 94	Controls' follow-up time *N* = 30
Time, *year*	Mean; median SD; range	6.5; 6.0 3.1; 1.0 to 15.1	5.4; 4.2 4.1; 1.1 to 16.0

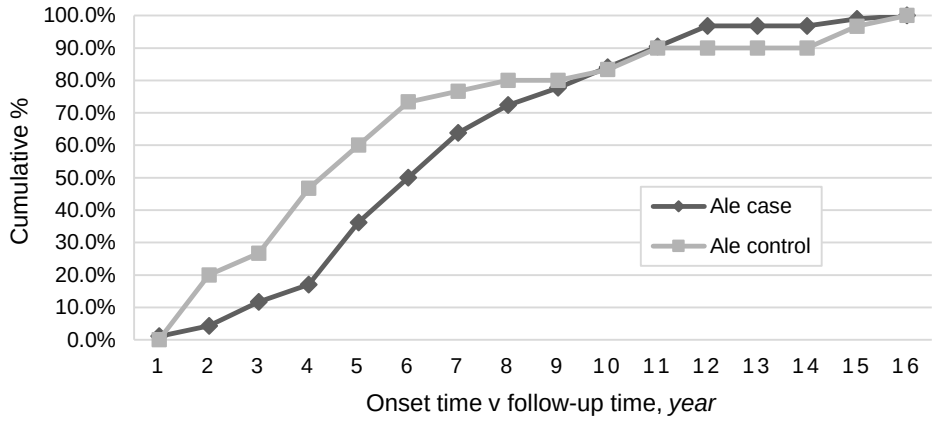
Graph 6. Alendronate cohort onset time versus follow-up time; number of individuals over time



Year, *n*th	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Ale case, *n*	1	3	7	5	18	13	13	8	5	6	6	6	0	0	2	1
Ale control, *n*	0	6	2	6	4	4	1	1	0	1	2	0	0	0	2	1

Ale = Alendronate

Graph 7. Alendronate cohort onset time versus follow-up time; cumulative percentage over time



Year, *n*th	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Ale case, *%*	1.1	4.3	11.7	17.0	36.2	50.0	63.8	72.3	77.7	84.0	90.4	96.8	96.8	96.8	98.9	100.0
Ale control, *%*	0.0	20.0	26.7	46.7	60.0	73.3	76.7	80.0	80.0	83.3	90.0	90.0	90.0	90.0	96.7	100.0

Ale = Alendronate

Table 20. Onset time versus follow-up time; random-effects univariable linear regression

case=0, control=1	*N*	Estimated coefficient	95% CI	*p*-value
All cases v All controls	629	-0.82	-1.34 to -0.31	0.002*
Zol cases v Zol controls	415	-0.48	-0.91 to -0.05	0.030*
Ale cases v Ale controls	124	-1.05	-2.41 to 0.31	0.129

* Significant results, $p<0.05$

```
Random-effects ML regression                    Number of obs      =       629
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      89.9
                                                               max =       498

                                                LR chi2(1)         =      9.05
Log likelihood  = -1620.2684                    Prob > chi2        =    0.0026

------------------------------------------------------------------------------
        time |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
case0control1 |  -.8244194   .2621592    -3.14   0.002    -1.338242   -.3105968
       _cons |    4.17588   .2393315    17.45   0.000     3.706799    4.644961
-------------+----------------------------------------------------------------
     /sigma_u |   .2871645          .                            .           .
     /sigma_e |    3.17442   .0897221                      3.00335    3.355234
          rho |    .008117          .                            .           .
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.66 Prob>=chibar2 = 0.209




Random-effects ML regression                    Number of obs      =       415
Group variable: zolcountry                      Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         1
                                                               avg =      59.3
                                                               max =       357

                                                LR chi2(1)         =      4.67
Log likelihood  =  -923.9201                    Prob > chi2        =    0.0307

------------------------------------------------------------------------------
        zoltime |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
----------------+-------------------------------------------------------------
zolcase0control1 |  -.4769792   .2201609    -2.17   0.030    -.9084867   -.0454718
          _cons |   2.850905     .15398    18.51   0.000     2.549109      3.1527
----------------+-------------------------------------------------------------
       /sigma_u |          0   .1183707                            .           .
       /sigma_e |   2.242015   .0778186                      2.094565    2.399844
            rho |          0  (omitted)
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.00 Prob>=chibar2 = 1.000




Random-effects ML regression                    Number of obs      =       124
Group variable: alecountry                      Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         2
                                                               avg =      17.7
                                                               max =        77

                                                LR chi2(1)         =      2.28
Log likelihood  = -324.21379                    Prob > chi2        =    0.1307

------------------------------------------------------------------------------
        aletime |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
----------------+-------------------------------------------------------------
alecase0control1 |  -1.052529    .693214    -1.52   0.129    -2.411203    .3061455
          _cons |   6.474264   .3409707    18.99   0.000     5.805974    7.142554
----------------+-------------------------------------------------------------
       /sigma_u |          0    .340432                            .           .
       /sigma_e |   3.305834   .2099206                      2.918971     3.74397
            rho |          0  (omitted)
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.00 Prob>=chibar2 = 1.000
```

4.4 Discussion

4.4.1 Main findings and comparison with previous studies

4.4.1.1 Time to onset

In the current cohort, the onset time amongst cases on zoledronate was shorter than those on alendronate. This has also been shown in a number of studies (Bamias et al., 2005; Mavrokokki et al., 2007; Pozzi et al., 2007; Boonyapakorn et al., 2008; Vahtsevanos et al., 2009; Thumbigere-Math et al., 2012; Watters et al., 2012) and can be explained by the higher potency of zoledronate (Dunford et al., 2001).

The median has been commonly calculated for time to onset. The median for the current zoledronate case cohort was 2.1 years. This is similar to the weighted average of 21.9 months (1.8 years) calculated in a previous review (Palaska et al., 2009), and a recent study of 109 ONJ cases associated with zoledronate and pamidronate, which reported a median onset time of 26.3 months (2.2 years) (Watters et al., 2012). However, results from earlier, smaller studies were different. Mavrokokki et al., 2007, a study on a small group of zoledronate cases amongst a cohort of 59 patients, reported a median onset time of 12 months. While Pozzi et al., 2007 calculated a median of 36 months amongst 35 cases on zoledronate and pamidronate.

As for cases on alendronate, their median time to onset was 6.0 years. This is longer than the weighted average of 4.6 years reported in Palaska et al., 2009, and nearly two times longer than findings from Mavrokokki et al., 2007, which studied a small group of alendronate cases amongst a cohort of 59 patients and reported a median of two years.

For the first time, the peak time of diagnosis and cumulative percentages of ONJ cases being diagnosed over time were analysed. For zoledronate cases, their peak time of diagnosis was in the second year. Whereas for the alendronate cohort, its peak time was in the fifth year. It took about four to five years for the vast majority, i.e. 80% cumulatively, of ONJ cases to be diagnosed amongst individuals prescribed with zoledronate. Again, it took about twice longer, i.e. nine to 10 years, to capture the majority of ONJ cases on alendronate.

4.4.1.2 Follow-up time

It is not possible to compare the follow-up time of the current control cohort with previous case-control studies as none reported the follow-up time of their controls recruited.

Meanwhile, it would be inappropriate to compare follow-up time with BPs duration or study period as follow-up time is defined precisely as time elapsed between initiation of BPs therapy and the latest follow-up.

4.4.1.3 Super-controls selection

This was an original and novel approach. After careful consideration of the onset time median, peak time of diagnosis, and yearly cumulative percentages, the median was chosen as the cut-off for choosing super-controls. Using this criterion, 88 zoledronate super-controls were chosen for additional analysis. Using the same criterion in the alendronate cohort, eight super-controls were selected. Since the number is too small for comparison with 94 alendronate cases, additional analysis will not follow.

4.4.2 Study strengths

4.4.2.1 Clear definition of time to onset and follow-up time

An important strength of this part of the study is its clear definition of time to onset and follow-up time. Onset time has been precisely defined as the period from the initiation of BPs therapy to ONJ diagnosis. Clear diagnostic criteria for ONJ have also been adopted, so as to ensure consistency across clinicians and clinical centres in this multicentre study. In contrast, previous studies did not differentiate between ONJ diagnosis by clinicians from report of symptoms by patients, which is prone to inconsistency as well as errors associated with misreporting of ONJ manifestations by patients (Marx et al., 2005; Boonyapakorn et al., 2008; Lazarovici et al., 2009).

The follow-up time has also been clearly defined. It refers to the period between the initiation of BPs therapy and patient recruitment, which differentiates follow-up time from cumulative duration of BPs therapy. In fact, unlike follow-up time, BPs duration is not comparable with cases' time to onset since the end date of BPs therapy does not always coincide with ONJ diagnosis for cases, or the latest follow-up for controls. Therefore, it is not ideal to validate a control cohort through comparison between duration of BPs therapy and time to onset.

4.4.2.2 Detailed analysis

Both descriptive and inferential statistics have been carefully performed. Apart from testing if time to onset and follow-up time were statistically significantly different from each other, year by year comparison of the number of cases being diagnosed and controls having been reviewed was also performed. This has been an original and in-depth approach in providing more information in

addition to the regression analysis. As demonstrated in the zoledronate cohort, $p<0.05$ does not necessarily mean that there was a large difference between cases' onset time and controls' follow-up time. In fact, the medians only differed by 0.4 years and their distribution was found mostly similar to each other. The significant difference was due mainly to 57, out of a total of 203 controls, who had only been reviewed for under a year.

In contrast, as for the alendronate cohort, although the median follow-up time was by nearly two years shorter than that of the onset time, probably due to the small cohort size of the controls, their difference was not found statistically significant.

4.4.2.3 Novel super-controls selection

In the current study, different control cohort validation approaches have been considered.

The first option was to compare the current cohort's follow-up time with previous studies' time to onset. However, there is no single up-to-date figure available as the most recent review study was published by Palaska et al., 2009. Moreover, there is a wide range of time to onset figures reported. As in the case of zoledronate, it ranged widely from 0.5 to six years (Palaska et al., 2009). Therefore, it was decided to compare the current cohort's follow-up time with its own time to onset. This is considered more favourable as the participants were recruited by the same consortium under the same setting. In addition, the current time to onset figure was found comparable with a recent study of 109 ONJ cases (Watters et al., 2012).

An alternative approach also considered was to accept or reject the entire control cohort, on the basis of any statistically significant differences between time to onset and follow-up time. If this approach were chosen, the alendronate control cohort would have been considered “acceptable”, although the majority had a review period shorter than the median onset time and peak time of diagnosis. On the other hand, the entire zoledronate control cohort would have been rejected as its follow-up time was statistically significantly shorter than its time to onset. However, there were in fact 88 zoledronate super-controls.

It is important to achieve the right balance in choosing the appropriate controls and is probably more sensible to select part of the cohort who were super-controls, through careful comparison between follow-up time and time to onset, with reference to different evidences, including their medians and numbers of (i) cases being diagnosed, and (ii) controls being reviewed at different lengths of time, as performed in the current study.

4.4.3 Study limitations

Study limitations were mainly missing data, small cohort size amongst those on alendronate and other BPs, and their related issues.

Time-related data were incomplete in 36 cases (9.2%) and four controls (1.4%), making the calculation of time amongst these individuals impossible.

The alendronate cohort size was small and there were only 31 controls, versus 109 cases, making the selection of super-controls and additional risk factor analysis impossible. However, a recent study only recruited 98 alendronate patients in total, which is by 42 patients less than the current alendronate cohort (Sedghizadeh et al., 2013).

In the GENVABO cohort, there were even fewer participants managed with other types of BPs. For instance, there were only 22 cases and 17 controls managed with pamidronate, followed by 15 cases and seven controls managed with ibandronate. Such cohort sizes are too small for stratification and further analysis. This is however understandable, as zoledronate is the most frequently prescribed intravenous BPs and alendronate for oral BPs (Filleul et al., 2010).

Currently, the median was chosen as the threshold in selecting super-controls. If more controls were available, an even more stringent criterion, e.g. 80% cumulatively, could be used instead, which would further lower the risk of including “false-controls” for further analysis.

4.5 Links to Chapter 5

The time to onset median amongst zoledronate cases was 2.1 years and was chosen as the cut-off for selecting super-controls. Eighty eight zoledronate super-controls, having been reviewed for 2.1 years or longer, were selected and will be subject to additional risk factor analysis in Chapter 5. Meanwhile, there were only eight super-controls in the alendronate cohort. Since the number is too small for comparison with 94 alendronate cases, additional risk factor analysis will not follow. Of note, genotyping of these BPs-exposed controls is still in progress and they are currently not involved in the GWAS in Chapter 6.

CHAPTER 5

Clinical Risk Factors

ONJ cases versus BPs-exposed controls

5.1 Introduction

5.1.1 Literature review

ONJ is considered a multifactorial disease and a number of risk factors have been suggested (Landesberg et al., 2011). The investigation of these clinical factors in the current GENVABO GWAS is also important as there may be clinical-genetic interaction involved in the pathogenesis of ONJ (Izzotti et al., 2013).

To date, there have been several studies reporting risk factors for ONJ development. However, only eight investigated the combined effect of these factors through multivariable statistics (Table 21). Nonetheless, there remained a number of issues with respect to their design and methodology.

Five studies considered BPs dosage or duration when recruiting controls (Kyrgidis et al., 2008; Vahtsevanos et al., 2009; Thumbigere-Math et al., 2012, 2013; Sedghizadeh et al., 2013). However, their requirements were not ideal. For instance, the minimum BPs dosage requirement in Thumbigere-Math et al., 2012 was one dose only, while the minimum BPs therapy duration requirement in Sedghizadeh et al., 2013 was only three months. In fact, none screened specifically the follow-up time of the controls. Therefore, as explained in the previous Chapter, they may carry a risk of recruiting “false-controls”.

Another issue was with the size of their cohorts. Out of all eight studies, four recruited less than 100 participants (Kyrgidis et al., 2008; Katz et al., 2011; Thumbigere-Math et al., 2013; Tsao et al., 2013). On the other hand, for those

with a larger cohort, their case cohorts remained small. For instance, Thumbigere-Math et al., 2012 recruited only 18 cases for comparison with 558 controls. In fact, none of the eight studies had a case cohort larger than 100. This indicates that these studies can provide only a narrow representation of ONJ phenotype.

Furthermore, there was no cohort stratification according to BPs type in any of the studies. For instance, zoledronate and alendronate have very different potency, indication, ONJ incidence and time to ONJ onset. However, in two studies, individuals on zoledronate and alendronate were analysed together (Wessel et al., 2008; Sedghizadeh et al., 2013). As for zoledronate and pamidronate, although both are usually prescribed intravenously, their ONJ incidences were reported as 0-20% and 0-4% respectively (Kühl et al., 2012), and their difference in time to onset was almost a year (Palaska et al., 2009). However, most studies did not differentiate zoledronate patients from those on pamidronate, and some included ibandronate patients as well (Kyrgidis et al., 2008; Vahtsevanos et al., 2009; Katz et al., 2011; Thumbigere-Math et al., 2012, 2013). Although this boosted the overall cohort sizes, it may risk inappropriate and irrelevant comparison of cases and controls on different types of BPs. In other words, it is not desirable to compare, for example, a case on alendronate with a control on zoledronate, as they would also be very different with regard to underlying diseases, BPs dosage and concomitant medications.

Possibly due to these limitations, current evidences on ONJ clinical risk factors remained controversial and were largely unhelpful in understanding the pathophysiology of ONJ (Campisi et al., 2014).

Table 21. Previous studies on factors for ONJ development using multivariable technique

Study	Case, *n*	Case inclusion	Control, *n*	Control inclusion	Underlying disease	BPs type	Risk factors	Protective factors
Sedghizadeh et al. 2013*	69	AAOMS Stage 0 to 3	84	Minimum BPs duration: 3 months	Cancer, Osteoporosis	Alendronate (Ale), Ibandronate (Iba), Pamidronate (Pam), Risedronate (Ris), Zoledronate (Zol)	- Longer duration BPs therapy - Older age - Asian race	/
Thumbigere-Math et al. 2013*	25	AAOMS definition	48	Minimum i.v. BPs dose: 10	Breast cancer, Lung cancer, Multiple myeloma, Prostate cancer, Renal cell carcinoma	Zol, Pam	- Periodontal disease	/
Tsao et al. 2013*	22	ASBMR definition	41	Matched age and gender	Breast cancer, Multiple myeloma	i.v. BPs	- Periodontal disease	/
Thumbigere-Math et al., 2012*	18	AAOMS definition	558	Minimum i.v. BPs dose: 1	Breast cancer, Lung cancer, Multiple myeloma, Prostate cancer, Renal cell carcinoma	Zol, Pam	- Diabetes, - Smoking, - Steroids, - Hypothyroidism, - Pam infusion, - Zol infusion	/
Katz et al. 2011*	12	AAOMS definition	66	/	Multiple myeloma	Zol, Pam	- Smoking	/
Vahtsevanos et al. 2009*	80	AAOMS definition	1541	Minimum BPs duration: 5 months	Breast cancer, Multiple myeloma, Prostate cancer	Zol, Pam, Iba	- Each dose of Zol, Pam administered, - Ever received Zol, Pam, - Denture, - Extraction	- Each dose of all BPs administered, - Ever received Iba
Wessel et al. 2008*	30	International classification of disease (ICD-9) diagnostic code	150	Matched age, gender, cancer type and year of cancer diagnosis	Breast cancer, Kidney cancer, Lung cancer, Multiple myeloma, Prostate cancer	Zol, Pam, Oral BPs	- Smoking, - Obesity, - Zol	/
Kyrgidis et al., 2008*	20	Developed ONJ	40	1 less to 3 more doses of BPs than cases; matched age	Breast cancer	Zol, Pam, Iba	- Extraction - Denture	/

* No cohort stratification according to BPs type.

5.1.2 Objectives

The objective is to investigate ONJ clinical risk factors, in a large, multicentre cohort, coupled with cohort stratification according to BPs type and ONJ type, as well as with super-controls, all analysed using multivariable statistics**.**

5.2 Methods

This part of the study consists of a pre-analysis, the main risk factor analysis, followed by a post-hoc analysis. Information related to clinical risk factors was recorded in the CRF, then transferred into electronic spreadsheets. All statistical analyses were performed in Stata version 12.1 (Stata Corp., College Station, TX, US).

5.2.1 Analysis cohorts

In addition to the overall cohort which included all cases and controls, analyses were carried out in five more stratified cohorts (Table 22). Stratified cohorts were limited to zoledronate and alendronate only because others were much smaller, for example, the pamidronate cohort had 39 individuals only.

There were cohorts with the exposed type ONJ cases, managed with zoledronate and alendronate respectively. As discussed in Chapter 4, there was also the zoledronate super-controls cohort. However, the number of the non-exposed cases (zoledronate: *N*=18; alendronate: *N*=16), as well as the alendronate super-controls (*N*=18), were too small, hence, there would not be analyses for these small groups.

Risk factor analyses were carried out for each respective cohort and their results were summarised and compared with each other.

Table 22. Analysis cohorts

Cohorts	Overall cohort	Zoledronate all cases and all controls	Zoledronate exposed type cases and all controls	Zoledronate all cases and super-controls	Alendronate all cases and all controls	Alendronate exposed type cases and all controls
Cases, *n*	393	230	208	230	109	89
Controls, *n*	276	204	204	88	31	31
Total	669	434	412	318	140	120

5.2.2 Pre-risk factor analysis

Prior to risk factor analysis, there was pre-analysis to study the differences across different cohorts. Phenotypic features of (i) the zoledronate users against the alendronate users, and (ii) the zoledronate super-controls against controls reviewed for less than 2.1 years were compared. Of note, ONJ types had already been compared in Chapter 3 and were found largely phenotypically similar.

This pre-analysis served to explain the final ONJ risk factor results.

5.2.2.1 Outcomes

5.2.2.1.1 Primary outcome

The primary aim was to make comparisons across different cohorts using descriptive statistics. The primary objective was to detect if there were any major differences with respect to their phenotype data.

5.2.2.1.2 Secondary outcome

The secondary aim was to make comparisons across the cohorts using inferential statistics. The objective was to detect if there were any phenotypically, statistically significant differences between different cohorts.

5.2.2.2 Statistical analysis

5.2.2.2.1 Primary outcome

In both comparisons, mean, median, standard deviation and range were calculated for numerical phenotypic data, while numbers and percentages were calculated for categorical data. The percentages were also plotted in a bar chart, constructed in Microsoft Excel 2013.

5.2.2.2.2 Secondary outcome

When comparing the two BPs types, the explanatory variable was zoledronate user=1, while alendronate user=0. As for control status, its explanatory variable was super-control=1 and control reviewed <2.1 years=0. Each phenotypic feature formed the outcome variable.

For numerical outcome variables, random-effects linear regression was performed, and random-effects logistic regression for binary phenotypic data. The significance level for these analyses was 5%.

Multilevel random-effects were used to account for the clustering effect attributed to the participants being recruited in seven countries.

5.2.3 Risk factor analysis

5.2.3.1 Outcomes

5.2.3.1.1 Primary outcome

The primary aim was to investigate the association between ONJ development and its previously reported potential risk factors, as discussed in Chapter 1, using descriptive statistics. The primary objective was to report the prevalence of these factors amongst the ONJ cases and BPs-exposed controls.

5.2.3.1.2 Secondary outcome

The secondary aim was to investigate the association between ONJ development and potential risk factors, using inferential statistics. The objective was to detect if there were any statistically significant factors.

5.2.3.2 Statistical analysis

5.2.3.2.1 Primary outcome

The prevalence of each potential risk factor amongst the cases and the controls was studied. Their numbers and percentages were calculated; the percentages were also presented in a bar chart. For numerical data including age and BPs duration, their mean, median, standard deviation and range were calculated.

5.2.3.2.2 Secondary outcome

The association between ONJ development and each potential risk factor was first investigated using random-effects univariable logistic regression. ONJ development, i.e. case=1 and control=0, was the outcome variable, while each factor formed the explanatory variable. In total, 12 factors related to demographics, BPs history and medical history were analysed.

Factors that were significant at the 10% level in the univariable analysis were then entered together into a random-effects multivariable logistic regression, using a 5% significance level. Multilevel random-effects were used in both the univariable and multivariable analyses to account for the clustering effect attributed to the participants being recruited in seven countries.

5.2.4 Post-hoc analysis

5.2.4.1 Outcomes

5.2.4.1.1 Primary outcome

The primary aim was to study the interrelationship amongst the significant factors identified in the current risk factor analysis, using descriptive statistics. The primary objective was to detect if there was any strong interrelationship between the factors.

5.2.4.1.2 Secondary outcome

The secondary aim was to study the interrelationship amongst the significant factors using inferential statistics. The secondary objective was to detect if there was any statistically significant association between any of these factors.

5.2.4.2 Statistical analysis

5.2.4.2.1 Primary outcome

Two factors were studied at a time. For numerical factors data, their mean, median, standard deviation and range were calculated, while numbers and percentages were calculated for categorical data.

5.2.4.2.2 Secondary outcome

Similarly, two factors were studied at a time. For numerical variables, univariable linear regression was applied, and univariable logistic regression for binary variables. Multilevel random-effects were also used to account for the clustering effect attributed to the participants being recruited in seven countries. The significance level for these analyses was also 5%.

5.3 Results

5.3.1 Pre-risk factor analysis

5.3.1.1 Zoledronate users versus alendronate users

There were 434 zoledronate users, including both cases and controls, as well as 140 alendronate users (Table 23).

On average, those who had zoledronate were by approximately four years younger than the alendronate users. As for BPs duration, zoledronate therapy was by about four years shorter than that of alendronate. For the categorical phenotypic data, the percentages calculated were also shown in a bar chart (Graph 8). The major differences were with underlying diseases, ranging from 9.0 to 92.2%, followed by gender proportion (female) (32.9%), then with use of antiangiogenics (24.7%).

It was also confirmed that the zoledronate users were statistically significantly younger than the alendronate users (estimated coefficient -0.49 decades, 95% CI -0.68 to -0.29 decades, $p<0.001$) (Table 24). Zoledronate therapy was also statistically significantly shorter than that of alendronate (estimated coefficient -3.62 years, 95% CI -4.10 to -3.15 years, $p<0.001$).

As for underlying diseases, the zoledronate group had a larger proportion of multiple myeloma and metastatic breast cancer patients than the alendronate group, both statistically significant (multiple myeloma: OR=48.44, 95% CI 11.84 to 198.21, $p<0.001$; metastatic breast cancer: OR=9.87, 95% CI 4.25 to 22.93, $p<0.001$). The zoledronate group also had more antiangiogenics users, also statistically significant (OR=9.87, 95% CI 4.25 to 22.93, $p<0.001$).

In contrast, the proportion of osteoporosis patients amongst the zoledronate users was statistically significantly smaller than in the alendronate group (OR=0.001, 95% CI 0.000 to 0.003, $p<0.001$).

As for diabetes, smoking history and use of steroids, these were not found statistically significantly different between the two groups ($p>0.05$).

Of note, as none of the alendronate users were presented with metastatic prostate cancer or other cancers, these two variables could not be analysed with logistic regressions.

5.3.1.2 Zoledronate super-controls versus controls reviewed for less than 2.1 years

There were 88 super-controls while 115 zoledronate controls were reviewed for less than 2.1 years (Table 25).

The two groups shared similar age, gender proportion, and the presence of systemic factors. On average, the super-controls were by approximately one to two years younger than the other controls. Their difference in the proportion of female patients was of 5.8% only. For the systemic factors, difference in proportion ranged from 0.4 to 11.8% (Graph 9).

However, there were 15.4% more multiple myeloma patients, but 11.0% less metastatic prostate cancer patients amongst the super-controls than in the other controls. Also, on average, the length of zoledronate therapy was by 2.13 years longer amongst the super-controls than in controls reviewed for less than 2.1 years.

With the regression analysis, the two groups' difference was not statistically significant with regard to age, gender, metastatic cancers, as well as diabetes, smoking, use of steroids and antiangiogenics (p>0.05) (Table 26). In particular, for age, the estimated coefficient was nearly zero, indicating that the super-controls and the rest of the controls shared very similar age.

However, there was a higher proportion of multiple myeloma patients amongst the super-controls (OR=1.87, 95% CI 1.06 to 3.28, p=0.030). Compared to the other controls, BPs duration of super-controls was by approximately two years longer (estimated coefficient 2.13 years, 95% CI 1.74 to 2.52 years, p<0.001).

5.3.1.3 Summary

It was found that the zoledronate and alendronate users were different in many aspects, including age, gender, underlying diseases and BPs duration. Chapter 3 also reported that the zoledronate and alendronate cases had different proportion of individuals presented with different ONJ types. In addition, Chapter 4 found that the two groups had different ONJ time to onset and follow-up time amongst the cases and controls respectively. Therefore, in the subsequent ONJ risk factor analysis, it is necessary to perform cohort stratification according to BPs type.

As for the zoledronate super-controls and controls reviewed for less than 2.1 years, they were found largely phenotypically similar, except mainly that the super-controls had longer BPs duration. With a dedicated super-control cohort and an all controls cohort, the contribution of super-control selection towards ONJ risk factor analysis could then be investigated, through follow-up time screening and BPs duration adjustment.

Table 23. Zoledronate users versus alendronate users; descriptive statistics

		Zoledronate users *N* = 434		Alendronate users *N* = 140	
		n	**%**	***n***	**%**
Age, *decade*	Mean, median	6.7, 6.8		7.1, 7.2	
	SD	1.1		1.0	
	Range	3.5 to 8.8		3.7 to 8.9	
Gender	Female	251	57.8%	127	90.7%
	Male	180	41.5%	13	9.3%
Primary underlying disease	Multiple myeloma	179	41.2%	2	1.4%
	Osteoporosis	9	2.1%	132	94.3%
	Metastatic breast cancer	133	30.6%	6	4.3%
	Metastatic prostate cancer	74	17.1%	0	0.0%
	Other cancers	39	9.0%	0	0.0%
BPs duration, *year*	Mean, median	2.2, 1.6		5.9, 5.3	
	SD	2.0		3.3	
	Range	0.1 to 12.1		0.1 to 16.0	
Systemic factor	Diabetes	36	8.3%	14	10.0%
	Smoking (no=0, yes/ex=1)	90	20.7%	29	20.7%
	Steroids	76	17.5%	32	22.9%
	Antiangiogenics	126	29.0%	6	4.3%

Graph 8. Zoledronate users versus alendronate users; differences in percentages

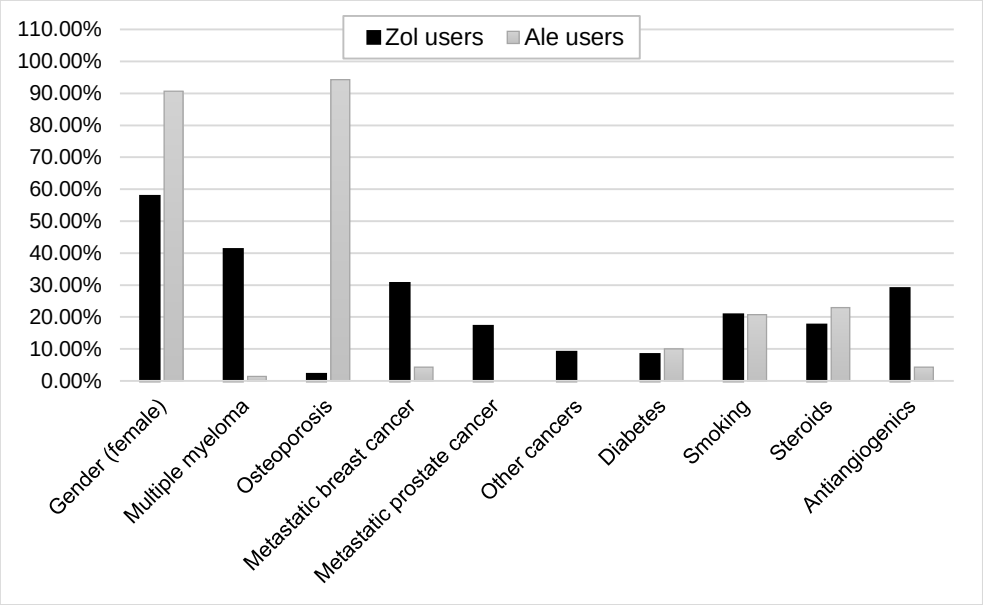


Table 24. Zoledronate users versus alendronate users; random-effects univariable regression

		***N* = 574; zol user=1, ale user=0**				
		Estimated coefficient	**95% CI**			***p*-value**
Demographics	Age, *decade*	-0.49	-0.68	to	-0.29	<0.001*
BPs duration, *year*		-3.62	-4.10	to	-3.15	<0.001*
		OR	**95% CI**			***p*-value**
Demographics	Gender (M=0, F=1)	0.14	0.08	to	0.26	<0.001*
Primary underlying disease	Multiple myeloma	48.44	11.84	to	198.21	<0.001*
	Osteoporosis	0.001	0.000	to	0.003	<0.001*
	Metastatic breast cancer	9.87	4.25	to	22.93	<0.001*
Systemic factor	Diabetes	0.85	0.43	to	1.65	0.623
	Smoking (no=0, yes/ex=1)	0.92	0.57	to	1.50	0.743
	Steroids	0.87	0.52	to	1.45	0.600
	Antiangiogenics	8.23	3.49	to	19.39	<0.001*

* Significant results, $p<0.05$

```
Random-effects ML regression                    Number of obs      =       573
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      81.9
                                                               max =       439

                                                LR chi2(1)         =     23.20
Log likelihood  = -828.93201                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
   agedecade |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     ale1zol |  -.4854701   .0999552    -4.86   0.000    -.6813788   -.2895615
       _cons |   7.131429   .0868905    82.07   0.000     6.961126    7.301731
-------------+----------------------------------------------------------------
    /sigma_u |   1.38e-23   .0551672                             0           .
    /sigma_e |   1.028102   .0303699                      .9702682    1.089383
         rho |   1.80e-46   1.44e-24                             0           1
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.00 Prob>=chibar2 = 1.000




Random-effects ML regression                    Number of obs      =       553
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      79.0
                                                               max =       436

                                                LR chi2(1)         =    187.61
Log likelihood  = -1252.9492                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
durationyearplus1mo |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------
            ale1zol |  -3.623507   .2414999   -15.00   0.000    -4.096838   -3.150176
              _cons |   5.908647   .2627249    22.49   0.000     5.393716    6.423579
--------------------+---------------------------------------------------------
           /sigma_u |   .3369396    .318299                      .0528983    2.146161
           /sigma_e |    2.32325   .0705365                      2.189034    2.465695
                rho |   .0206002   .0382695                      .0001909    .2978102
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.65 Prob>=chibar2 = 0.209
```

```
Random-effects logistic regression              Number of obs      =       571
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      81.6
                                                               max =       437

                                                Wald chi2(1)       =     40.18
Log likelihood  = -336.14551                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
        m0f1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     ale1zol |   .1427345   .0438386    -6.34   0.000     .0781801    .2605922
       _cons |   9.768454   2.844477     7.83   0.000     5.520322    17.28571
-------------+----------------------------------------------------------------
    /lnsig2u |  -15.49344   69.48694                     -151.6853    120.6985
-------------+----------------------------------------------------------------
     sigma_u |   .0004322   .0150147                      1.15e-33    1.62e+26
         rho |   5.68e-08   3.94e-06                      4.04e-67           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       574
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      82.0
                                                               max =       439

                                                Wald chi2(1)       =     29.13
Log likelihood  = -304.61971                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
          mm |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     ale1zol |   48.43764    34.8224     5.40   0.000     11.83707    198.2083
       _cons |   .0144904    .010321    -5.94   0.000     .0035875    .0585281
-------------+----------------------------------------------------------------
    /lnsig2u |  -14.86351   67.59564                     -147.3485    117.6215
-------------+----------------------------------------------------------------
     sigma_u |   .0005921   .0200133                      1.01e-32    3.48e+25
         rho |   1.07e-07   7.20e-06                      3.09e-65           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       574
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      82.0
                                                               max =       439

                                                Wald chi2(1)       =    160.31
Log likelihood  = -72.873583                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
          op |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     ale1zol |   .0012229   .0006478   -12.66   0.000      .000433    .0034536
       _cons |   38.16264   26.43574     5.26   0.000     9.817491    148.3462
-------------+----------------------------------------------------------------
    /lnsig2u |  -.1630237   1.044889                     -2.210969    1.884921
-------------+----------------------------------------------------------------
     sigma_u |   .9217218   .4815485                      .3310505    2.566288
         rho |   .2052382   .1704376                      .0322388    .6668725
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     3.16 Prob >= chibar2 = 0.038
```

```
Random-effects logistic regression              Number of obs      =       574
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      82.0
                                                               max =       439

                                                Wald chi2(1)       =     28.33
Log likelihood  = -292.21356                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
         mbc |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     ale1zol |   9.868222   4.244143     5.32   0.000     4.247687    22.92584
       _cons |   .0447761   .0186846    -7.44   0.000     .0197629    .1014479
-------------+----------------------------------------------------------------
    /lnsig2u |   -14.9814   78.29119                     -168.4293    138.4665
-------------+----------------------------------------------------------------
     sigma_u |   .0005583   .0218531                      2.67e-37    1.17e+30
         rho |   9.47e-08   7.42e-06                      2.16e-74           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       574
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      82.0
                                                               max =       439

                                                Wald chi2(1)       =      0.24
Log likelihood  = -169.44684                    Prob > chi2        =    0.6226

------------------------------------------------------------------------------
          dm |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     ale1zol |   .8457715   .2878785    -0.49   0.623     .4340366    1.648086
       _cons |   .1144833   .0372176    -6.67   0.000     .0605374    .2165015
-------------+----------------------------------------------------------------
    /lnsig2u |  -2.309598   2.270671                     -6.760031    2.140835
-------------+----------------------------------------------------------------
     sigma_u |   .3151209   .3577679                      .0340469    2.916597
         rho |   .0292996   .0645804                      .0003522    .7211126
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.30 Prob >= chibar2 = 0.291
```

```
Random-effects logistic regression              Number of obs      =       490
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      70.0
                                                               max =       358

                                                Wald chi2(1)       =      0.11
Log likelihood  = -271.57868                    Prob > chi2        =    0.7432

------------------------------------------------------------------------------
     smoking |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     ale1zol |    .922354   .2275339    -0.33   0.743     .5687431    1.495819
       _cons |   .3411765   .0733709    -5.00   0.000     .2238341    .5200345
-------------+----------------------------------------------------------------
    /lnsig2u |  -15.00241   78.63884                     -169.1317    139.1269
-------------+----------------------------------------------------------------
     sigma_u |   .0005524   .0217208                      1.88e-37    1.63e+30
         rho |   9.28e-08   7.29e-06                      1.07e-74           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       574
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      82.0
                                                               max =       439

                                                Wald chi2(1)       =      0.27
Log likelihood  = -274.77976                    Prob > chi2        =    0.6004

------------------------------------------------------------------------------
     steroid |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     ale1zol |   .8721095   .2278207    -0.52   0.600     .5226514    1.455224
       _cons |   .3629296   .1069439    -3.44   0.001     .2037043    .6466133
-------------+----------------------------------------------------------------
    /lnsig2u |  -1.440503   1.130699                     -3.656631    .7756259
-------------+----------------------------------------------------------------
     sigma_u |   .4866299   .2751159                      .160684    1.473754
         rho |   .0671478   .0708258                      .007787    .3976608
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     3.62 Prob >= chibar2 = 0.029




Random-effects logistic regression              Number of obs      =       574
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      82.0
                                                               max =       439

                                                Wald chi2(1)       =     23.22
Log likelihood  =  -285.3335                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
     antiang |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     ale1zol |   8.226492   3.597859     4.82   0.000     3.490932    19.38599
       _cons |    .035678   .0172043    -6.91   0.000     .0138657    .0918035
-------------+----------------------------------------------------------------
    /lnsig2u |  -2.038965   1.404621                     -4.791972    .7140426
-------------+----------------------------------------------------------------
     sigma_u |   .3607817   .2533808                      .0910828    1.429066
         rho |   .0380591   .0514241                      .0025154    .3830069
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     1.79 Prob >= chibar2 = 0.091
```

Table 25. Zoledronate super-controls versus controls reviewed for less than 2.1 years; descriptive statistics

		Zol super-controls *N* = 88		Zol other controls *N* = 115	
		n	**%**	***n***	**%**
Age, *decade*	Mean, median	6.4, 6.3		6.5, 6.5	
	SD	1.1		1.2	
	Range	3.5 to 8.4		3.6 to 8.8	
Gender	Female	51	58.0%	60	52.2%
	Male	37	42.0%	53	46.1%
Primary underlying disease	Multiple myeloma	48	54.5%	45	39.1%
	Osteoporosis	1	1.1%	2	1.7%
	Metastatic breast cancer	24	27.3%	31	27.0%
	Metastatic prostate cancer	11	12.5%	27	23.5%
	Other cancers	4	4.5%	10	8.7%
BPs duration, *year*	Mean, median	3.1, 2.4		0.9, 0.9	
	SD	2.1		0.5	
	Range	0.2 to 11.4		0.1 to 2.0	
Systemic factor	Diabetes	6	6.8%	1	0.9%
	Smoking (no=0, yes/ex=1)	18	20.5%	24	20.9%
	Steroids	17	19.3%	18	15.7%
	Antiangiogenics	41	46.6%	40	34.8%

Graph 9. Zoledronate super-controls versus controls reviewed for less than 2.1 years; differences in percentages

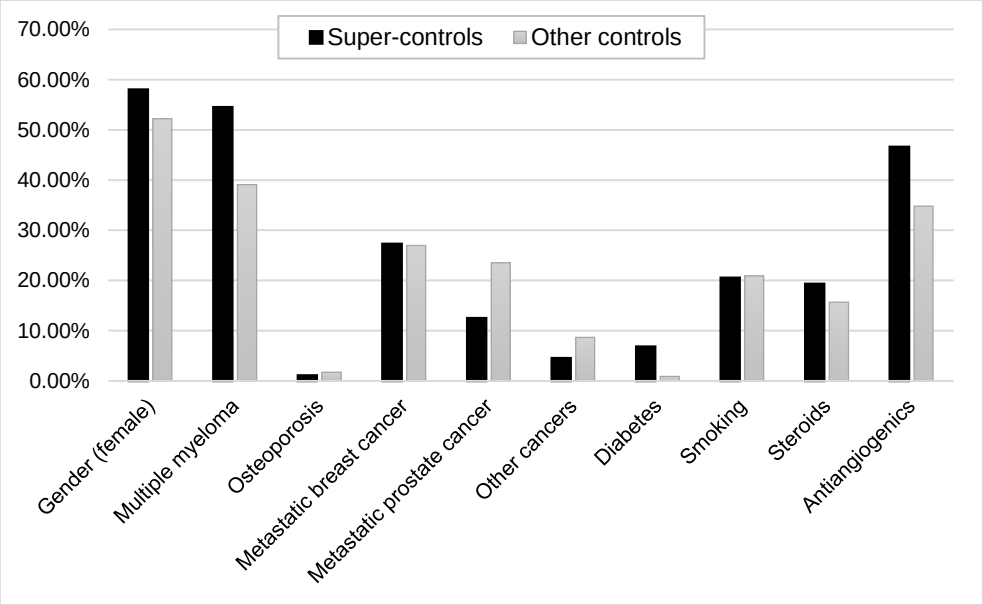


Table 26. Zoledronate super-controls versus controls reviewed for less than 2.1 years; random-effects univariable regression

		***N* = 203; super-control=1, control=0**					
		Estimated coefficient	**95% CI**				***p*-value**
Demographics	Age, *decade*	-0.05	-0.37	to	0.27		0.775
BPs duration, *year*		2.13	1.74	to	2.52		<0.001*
		OR	**95% CI**				***p*-value**
Demographics	Gender (M=0, F=1)	1.25	0.71	to	2.20		0.436
Primary underlying disease	Multiple myeloma	1.87	1.06	to	3.28		0.030*
	Osteoporosis	0.65	0.06	to	7.28		0.726
	Metastatic breast cancer	0.91	0.47	to	1.73		0.764
	Metastatic prostate cancer	0.47	0.22	to	1.00		0.050
	Other cancers	0.50	0.15	to	1.65		0.255
Systemic factor	Diabetes	0.86	0.29	to	2.52		0.786
	Smoking (no=0, yes/ex=1)	0.89	0.44	to	1.78		0.739
	Steroids	1.29	0.62	to	2.68		0.494
	Antiangiogenics	1.64	0.93	to	2.89		0.090

* Significant results, $p<0.05$

```
Random-effects ML regression                    Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                LR chi2(1)         =      0.08
Log likelihood  = -317.44673                    Prob > chi2        =    0.7749

------------------------------------------------------------------------------
    agedecade |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
supercontrol1 |  -.0467799   .1635825    -0.29   0.775    -.3673956    .2738358
        _cons |   5.926057   .5501489    10.77   0.000     4.847784    7.004329
--------------+---------------------------------------------------------------
     /sigma_u |   .6677328   .4280959                      .190056    2.345977
     /sigma_e |   1.140739   .0569315                     1.034439    1.257962
          rho |   .2551964   .2449862                     .0156613    .7985627
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    2.64 Prob>=chibar2 = 0.052
```

```
Random-effects ML regression                    Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                LR chi2(1)         =     91.85
Log likelihood  = -355.69217                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
durationyearplus1mo |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------
      supercontrol1 |   2.130059    .197631    10.78   0.000      1.74271    2.517409
              _cons |   .9313044   .1301213     7.16   0.000     .6762714    1.186337
--------------------+---------------------------------------------------------
           /sigma_u |          0    .101241                           .           .
           /sigma_e |   1.395482   .0692459                     1.266153     1.53802
                rho |          0  (omitted)
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.00 Prob>=chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       200
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         5
                                                               avg =     100.0
                                                               max =       195

                                                Wald chi2(1)       =      0.61
Log likelihood  =  -137.1129                    Prob > chi2        =    0.4363

------------------------------------------------------------------------------
         m0f1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
supercontrol1 |   1.251269    .360316     0.78   0.436     .7116035    2.200204
        _cons |   1.132278   .2134434     0.66   0.510     .7825189    1.638367
--------------+---------------------------------------------------------------
     /lnsig2u |  -15.22063   125.9873                     -262.1512    231.7099
--------------+---------------------------------------------------------------
      sigma_u |   .0004953   .0312017                      1.19e-57    2.07e+50
          rho |   7.46e-08   9.40e-06                      4.3e-115           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                Wald chi2(1)       =      4.73
Log likelihood  = -137.60553                    Prob > chi2        =    0.0296

------------------------------------------------------------------------------
           mm |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
supercontrol1 |   1.866884   .5357084     2.18   0.030     1.063806    3.276215
        _cons |   .6427944   .1228204    -2.31   0.021     .4420087    .9347884
--------------+---------------------------------------------------------------
     /lnsig2u |  -17.84907   131.3971                     -275.3827    239.6845
--------------+---------------------------------------------------------------
      sigma_u |   .0001331   .0087434                      1.59e-60    1.11e+52
          rho |   5.38e-09   7.07e-07                      7.7e-121           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                Wald chi2(1)       =      0.12
Log likelihood  = -15.557712                    Prob > chi2        =    0.7263

------------------------------------------------------------------------------
           op |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
supercontrol1 |   .6494178   .8007071    -0.35   0.726     .0579463    7.278182
        _cons |    .017701   .0126261    -5.66   0.000     .0043736    .0716409
--------------+---------------------------------------------------------------
     /lnsig2u |  -13.88066   130.7024                     -270.0527    242.2914
--------------+---------------------------------------------------------------
      sigma_u |    .000968   .0632568                      2.28e-59    4.10e+52
          rho |   2.85e-07   .0000372                      1.6e-118           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =  3.0e-06 Prob >= chibar2 = 0.499
```

```
Random-effects logistic regression              Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                Wald chi2(1)       =      0.09
Log likelihood  = -117.66111                    Prob > chi2        =    0.7636

------------------------------------------------------------------------------
         mbc |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
supercontrol1 |   .9053199   .2993924    -0.30   0.764     .4734856    1.731001
        _cons |   1.023834   1.064039     0.02   0.982     .1335371    7.849769
-------------+----------------------------------------------------------------
     /lnsig2u |    .324526   1.475223                     -2.566858     3.21591
-------------+----------------------------------------------------------------
      sigma_u |    1.17617   .8675561                      .2770856     4.99259
          rho |   .2960203   .3074251                       .022805    .8834035
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     1.86 Prob >= chibar2 = 0.087
```

```
Random-effects logistic regression              Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                Wald chi2(1)       =      3.84
Log likelihood  = -95.829741                    Prob > chi2        =    0.0501

------------------------------------------------------------------------------
         mpc |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
supercontrol1 |   .4655924   .1817129    -1.96   0.050     .2166687    1.000497
        _cons |   .3067543   .0674904    -5.37   0.000     .1993031    .4721363
-------------+----------------------------------------------------------------
     /lnsig2u |  -14.72752   125.3469                     -260.403     230.948
-------------+----------------------------------------------------------------
      sigma_u |   .0006338   .0397232                     2.85e-57    1.41e+50
          rho |   1.22e-07   .0000153                     2.5e-114           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                Wald chi2(1)       =      1.29
Log likelihood  =  -50.24736                    Prob > chi2        =    0.2554

------------------------------------------------------------------------------
          oc |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
supercontrol1 |         .5   .3047247    -1.14   0.255     .1514278    1.650951
        _cons |   .0952381   .0315185    -7.11   0.000     .0497864    .1821843
-------------+----------------------------------------------------------------
     /lnsig2u |  -15.06212   149.4056                     -307.8917    277.7674
-------------+----------------------------------------------------------------
      sigma_u |   .0005362   .0400533                     1.39e-67    2.07e+60
          rho |   8.74e-08   .0000131                     5.9e-135           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                Wald chi2(1)       =      0.07
Log likelihood  = -53.471739                    Prob > chi2        =    0.7858

------------------------------------------------------------------------------
           dm |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
supercontrol1 |   .8617989   .4716094    -0.27   0.786     .2948464     2.51893
        _cons |   .0848842   .0294747    -7.10   0.000     .0429794    .1676458
--------------+---------------------------------------------------------------
     /lnsig2u |  -14.26258   126.9207                     -263.0225    234.4973
--------------+---------------------------------------------------------------
      sigma_u |   .0007997   .0507485                      7.68e-58    8.33e+50
          rho |   1.94e-07   .0000247                      1.8e-115           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       184
Group variable: country                         Number of groups   =         1

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =       6.0
                                                               max =         6

                                                Wald chi2(1)       =      0.11
Log likelihood  =  -126.6014                    Prob > chi2        =    0.7386

------------------------------------------------------------------------------
supercontrol1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
      smoking |   .8884613   .3148638    -0.33   0.739     .4435875    1.779499
        _cons |    .844157   .1421902    -1.01   0.315     .6068011    1.174357
--------------+---------------------------------------------------------------
     /lnsig2u |  -14.35137   44.35143                     -101.2786    72.57583
--------------+---------------------------------------------------------------
      sigma_u |    .000765   .0169635                      1.02e-22    5.75e+15
          rho |   1.78e-07   7.89e-06                      3.15e-45           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                Wald chi2(1)       =      0.47
Log likelihood  = -93.084287                    Prob > chi2        =    0.4939

------------------------------------------------------------------------------
      steroid |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
supercontrol1 |    1.29028   .4807017     0.68   0.494      .621675    2.677961
        _cons |    .185516   .0476158    -6.56   0.000     .1121777    .3068008
--------------+---------------------------------------------------------------
     /lnsig2u |  -14.52757   124.3208                     -258.1918    229.1367
--------------+---------------------------------------------------------------
      sigma_u |   .0007005   .0435404                      8.60e-57    5.71e+49
          rho |   1.49e-07   .0000185                      2.2e-113           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       203
Group variable: country                         Number of groups   =         2

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =     101.5
                                                               max =       197

                                                Wald chi2(1)       =      2.88
Log likelihood  = -135.09266                    Prob > chi2        =    0.0896

------------------------------------------------------------------------------
      antiang |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
supercontrol1 |   1.635638   .4740535     1.70   0.090     .9267986    2.886617
        _cons |   .5333334   .1044209    -3.21   0.001     .3633647    .7828072
--------------+---------------------------------------------------------------
     /lnsig2u |  -16.82379   1097.172                     -2167.242    2133.594
--------------+---------------------------------------------------------------
      sigma_u |   .0002222   .1219007                             0           .
          rho |   1.50e-08   .0000165                             0           .
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

5.3.2 Risk factor analysis

5.3.2.1 Overall cohort

This cohort consisted of all 669 individuals, including 393 cases and 276 controls (Table 27). On average, the cases were by three to four years older than the controls, and their BPs duration was by about a year longer than the controls. The prevalence of other ONJ risk factors was also calculated (Table 27, Graph 10). The prevalence of use of antiangiogenics amongst the cases and the controls were 14.5% and 34.1% respectively, resulting in a difference of 19.6%. Other major differences were with underlying diseases, up to 17.1%, and about 15.4-16.5% for BPs type. The rest differed by less than 10%.

In the univariable random-effects logistic regression, the following explanatory variables were significant at the 10% level: age, multiple myeloma, zoledronate, BPs duration and use of antiangiogenics (Table 28). These factors were then entered together into a multivariable random-effects logistic regression. After adjusting for other covariates, three factors remained statistically significant at the 5% level (Table 29). First, an increase in age by 10 years was associated with an increase in the odds of ONJ development by 56% (95% CI 1.27 to 1.91, $p<0.001$). Second, an increase in BPs duration by a year was associated with an increase in the odds of having ONJ by 13% (95% CI 1.03 to 1.24, $p=0.009$). However, risk of ONJ was by 56% lower amongst those managed with antiangiogenics, than those who were not (95% CI 0.27 to 0.74, $p=0.002$).

Of note, gender, multiple myeloma and metastatic cancers, zoledronate, as well as potential systemic risk factors including diabetes, smoking history and use of steroids were all not statistically significant ($p>0.05$).

5.3.2.2 Zoledronate all cases and all controls

About 65% of the participants were managed with zoledronate and there were 230 cases and 204 controls in this cohort (Table 30).

Similar to the overall cohort, the zoledronate cases were also by approximately three to four years older than the controls. Cases' duration on zoledronate was by approximately 0.5 years longer than the controls. Between the two groups, the major difference in the prevalence of ONJ risk factors was with use of antiangiogenics (cases: 19.1%; controls: 40.2%) (Graph 11). The rest differed by less than 10%.

In the univariable random-effects logistic regression, explanatory variables that were significant at the 10% level are: age, other cancers, BPs duration and use of antiangiogenics (Table 31).

Similar to the previous model, age, BPs duration and use of antiangiogenics remained statistically significant in the multivariable logistic regression (p<0.05) (Table 32). Individuals who were older, or managed with a longer length of BPs had higher odds of having ONJ (age: adjusted OR=1.64, 95% CI 1.31 to 2.07, p<0.001; BPs duration: adjusted OR=1.14, 95% CI 1.01 to 1.28, p=0.029). Whereas antiangiogenics was again a protective factor (adjusted OR=0.37, 95% CI 0.21 to 0.63, p<0.001). After adjusting for other covariates, underlying diseases became statistically not significant (p>0.05).

5.3.2.3 Zoledronate exposed type cases and all controls

208 exposed type ONJ cases were compared with 204 controls who were also managed with zoledronate.

In the univariable regression, same variables which were significant at the 10% level in the previous zoledronate model were also significant in the current cohort (Table 33). They were age, other cancers, BPs duration and use of antiangiogenics. These variables were then entered together into a multivariable regression.

Age, BPs duration and use of antiangiogenics remained statistically significant (p<0.05) (Table 34). First, an increase in age by 10 years was associated with an increase in the odds of ONJ development by 63% (95% CI 1.29 to 2.07, p<0.001). Second, an increase in BPs duration by a year was associated with an increase in the odds of having ONJ by 16% (95% CI 1.03 to 1.30, p=0.017). Finally, antiangiogenics was again a protective factor (adjusted OR=0.38, 95% CI 0.22 to 0.67, p=0.001).

On the other hand, other potential risk factors including gender, underlying diseases, diabetes, smoking history and use of steroids were all not statistically significant (p>0.05).

5.3.2.4 Zoledronate all cases and super-controls

There were 230 cases and 88 super-controls in the current cohort (Table 35). Super-controls carry a lower risk of ONJ development, i.e. a lower risk of being "false-controls", and their details and selection criteria can be found in the previous chapter.

As reported in 5.3.1.2, the super-controls were found largely phenotypically similar to those who were reviewed for less than 2.1 years, except the super-controls were presented with a larger proportion of multiple myeloma patients, and were also on a significantly longer length of zoledronate therapy.

Therefore, BPs duration of the controls was adjusted through super-controls selection. In the final case-control multivariable random-effects logistic regression, the statistically significant factors identified were: age, other cancers and antiangiogenics (p<0.05) (Table 36).

Similar to the two previous zoledronate models, an increase in age by 10 years was associated with an increase in the odds of ONJ development by 72% (95% CI 1.26 to 2.34, p=0.001). Individuals who were on BPs for other cancers had 3.87 the odds of ONJ, compared to the multiple myeloma patients (95% CI 1.16 to 12.84, p=0.027). Lastly, once again, antiangiogenics was a protective factor (adjusted OR=0.27, 95% CI 0.14 to 0.53, p<0.001).

5.3.2.5 Alendronate all cases and all controls

The alendronate cohort had 109 cases versus 31 controls (Table 37). On average, the cases were by two to four years older than the controls, and their BPs duration was by about one to two years longer than the controls. The major differences in the prevalence of ONJ risk factors between the two groups were use of steroids and smoking (Graph 12). The prevalence of use of steroids amongst the cases and the controls were 28.4% and 3.2% respectively, resulting in a difference of 25.2%. As for history of smoking, 23.9% of cases and 9.7% of controls were current or previous smokers, resulting in a difference of 14.2%. The rest differed by less than 10%.

Of note, none of the alendronate patients were presented with metastatic prostate cancers and there were no alendronate controls presented with multiple myeloma. Therefore, statistically, it would not be possible to analyse underlying diseases as a risk factor in the following alendronate cohorts.

Similarly, as none of the alendronate controls were managed with concomitant antiangiogenics, due to statistical consideration, this variable would also be excluded. So, six factors including age, gender, alendronate duration, diabetes, smoking and use of steroids were analysed in the two alendronate cohorts.

In the univariable regressions, age, BPs duration and use of steroids were significant at the 10% level (Table 38). When entered together into the final multivariable random-effects logistic regression, only use of steroids remained statistically significant (p<0.05) (Table 39). Individuals who were on steroids had 9.61 the odds of ONJ development, compared to those who were not on the medication (95% CI 1.19 to 77.49, p=0.034).

Of note, age, gender, BPs duration, as well as potential systemic risk factors including diabetes and smoking history were all not statistically significant (p>0.05).

5.3.2.6 Alendronate exposed type cases and all controls

89 exposed type ONJ cases and 31 controls who were also managed with alendronate were analysed.

Very similar to the previous model in 5.3.2.5, age, BPs duration and the use of steroids were again significant at the 10% level in the univariable regressions (Table 40). In the final multivariable random-effects logistic regression, again, only use of steroids remained statistically significant (p<0.05) (Table 41). The odds of ONJ development amongst the steroids patients, versus those who were not, was 12.07 (95% CI 1.45 to 100.22, p=0.021).

Similarly, variables including age, gender, BPs duration, diabetes and smoking were all not statistically significant (p>0.05).

5.3.2.7 Summary

In general, the overall and the zoledronate cohorts shared similar results. The four overall and zoledronate cohorts all found age as a statistically significant risk factor and antiangiogenics a significant protective factor for ONJ (Table 42). BPs duration was also found a significant risk factor in the overall cohort, the zoledronate all cases and controls cohort and the zoledronate exposed type cases cohort, except in the zoledronate super-control cohort. However, other cancers was found a significant risk factor in the zoledronate super-control cohort.

The two alendronate cohorts had different results from the overall and the zoledronate cohorts. Only one risk factor, use of steroids, was identified.

Other potential risk factors, including gender, underlying diseases except other cancers, diabetes and smoking were not found statistically significant in the overall and all stratified cohorts.

Table 27. Overall cohort; descriptive statistics

		Cases *N* = 393		Controls *N* = 276	
		n	**%**	***n***	**%**
Age, *decade*	Mean, median	6.9, 7.0		6.6, 6.6	
	SD	0.9		1.1	
	Range	3.7 to 8.9		3.5 to 8.8	
Gender	Female	278	70.7%	168	60.9%
	Male	115	29.3%	101	36.6%
Primary underlying disease	Multiple myeloma	103	26.2%	107	38.8%
	Osteoporosis	137	34.9%	49	17.8%
	Metastatic breast cancer	89	22.6%	63	22.8%
	Metastatic prostate cancer	37	9.4%	38	13.8%
	Other cancers	27	6.9%	14	5.1%
BPs with longest duration	Zoledronate	230	58.5%	204	73.9%
	Alendronate	109	27.7%	31	11.2%
BPs duration, *year*	Mean, median	3.7, 2.8		2.6, 1.7	
	SD	3.1		2.8	
	Range	0.1 to 19.9		0.1 to 20.4	
Systemic factor	Diabetes	36	9.2%	19	6.9%
	Smoking	81	20.6%	47	17.0%
	Steroids	82	20.9%	42	15.2%
	Antiangiogenics	57	14.5%	94	34.1%

Graph 10. Overall cohort; differences in percentages

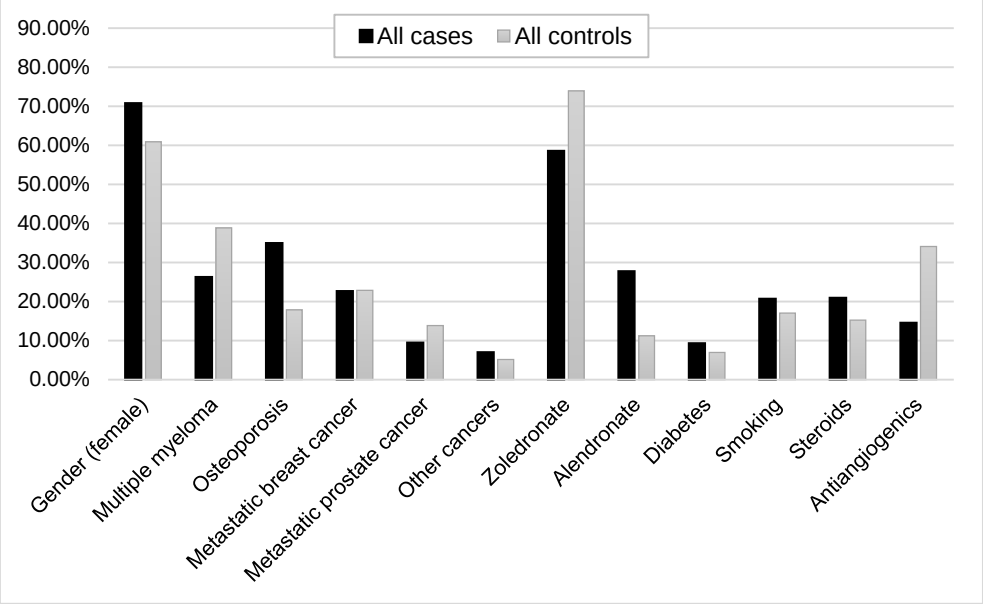


Table 28. Overall cohort risk factor analysis; random-effects univariable logistic regression

Variable		*N* = 669; case=1, control=0				
		OR	**95% CI**			***p*-value**
Demographics	Age, *decade*	1.48	1.25	to	1.76	<0.001
	Gender (M=0, F=1)	1.31	0.92	to	1.85	0.136
Primary underlying disease		**Osteoporosis (reference)**				
	Multiple myeloma	0.59	0.38	to	0.94	0.025
	Metastatic breast cancer	0.85	0.52	to	1.39	0.524
	Metastatic prostate cancer	0.61	0.34	to	1.11	0.106
	Other cancers	1.37	0.65	to	2.89	0.411
BPs with longest duration		**Alendronate (reference)**				
	Zoledronate	0.47	0.29	to	0.75	0.002
BPs duration, *year*		1.12	1.05	to	1.19	<0.001
Systemic factor	Diabetes	1.22	0.66	to	2.27	0.530
	Smoking (no=0, yes/ex=1)	1.32	0.85	to	2.05	0.216
	Steroids	1.20	0.77	to	1.87	0.421
	Antiangiogenics	0.39	0.26	to	0.58	<0.001

* Significant results, $p<0.1$

Table 29. Overall cohort risk factor analysis; random-effects multivariable logistic regression

Variable		*N* = 669; case=1, control=0				
		Adjusted OR	**95% CI**			***p*-value**
Demographics	Age, *decade*	1.56	1.27	to	1.91	<0.001*
Primary underlying disease		**Osteoporosis (reference)**				
	Multiple myeloma	1.10	0.32	to	3.72	0.881
	Metastatic breast cancer	1.19	0.37	to	3.83	0.767
	Metastatic prostate cancer	0.68	0.19	to	2.42	0.555
	Other cancers	2.44	0.62	to	9.52	0.199
BPs with longest duration		**Alendronate (reference)**				
	Zoledronate	0.83	0.26	to	2.67	0.759
BPs duration, *year*		1.13	1.03	to	1.24	0.009*
Systemic factor	Antiangiogenics	0.44	0.27	to	0.74	0.002*

* Significant results, $p<0.05$

```
Random-effects logistic regression              Number of obs      =       553
Group variable: Country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         6
                                                               avg =      79.0
                                                               max =       436

                                                Wald chi2(8)       =     48.32
Log likelihood  =  -313.7397                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
           control0case1 |          OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------------------+----------------------------------------------------------------
             agedecade |   1.555517   .1631779     4.21   0.000     1.266431    1.910593
                       |
diseaselop2mm3mbc4mpc5oc |
                     2  |   1.097612   .6832128     0.15   0.881     .3240525     3.71777
                     3  |   1.193194   .7104921     0.30   0.767     .3714154    3.833208
                     4  |   .6836885    .440533    -0.59   0.555     .1933698    2.417285
                     5  |   2.438939   1.694729     1.28   0.199     .6247873    9.520716
                       |
               ale1zol |   .8334261   .4950679    -0.31   0.759     .2601599    2.669893
    durationyearplus1mo |   1.130775    .053236     2.61   0.009     1.031104    1.240082
               antiang |   .4446622   .1146006    -3.14   0.002     .2683207     .736896
                 _cons |   .6257754   .7890183    -0.37   0.710      .052865    7.407451
-------------------------+----------------------------------------------------------------
              /lnsig2u |   .9894949   1.057461                     -1.083091    3.062081
-------------------------+----------------------------------------------------------------
               sigma_u |   1.640084   .8671625                      .5818483    4.622984
                   rho |   .4498312   .2617038                      .0933045    .8666009
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    44.82 Prob >= chibar2 = 0.000
```

Table 30. Zoledronate cohort; descriptive statistics

		Cases *N* = 230		Controls *N* = 204	
		n	**%**	***n***	**%**
Age, *decade*	Mean, median	6.8, 6.9		6.5, 6.5	
	SD	0.9		1.2	
	Range	3.7 to 8.8		3.5 to 8.8	
Gender	Female	139	60.4%	112	54.9%
	Male	91	39.6%	89	43.6%
Primary underlying disease	Multiple myeloma	85	37.0%	94	46.1%
	Osteoporosis	6	2.6%	3	1.5%
	Metastatic breast cancer	78	33.9%	55	27.0%
	Metastatic prostate cancer	36	15.7%	38	18.6%
	Other cancers	25	10.9%	14	6.9%
BPs duration, *year*	Mean, median	2.4, 1.8		1.9, 1.3	
	SD	2.1		1.8	
	Range	0.1 to 12.1		0.1 to 11.4	
Systemic factor	Diabetes	21	9.1%	15	7.4%
	Smoking	48	20.9%	42	20.6%
	Steroids	41	17.8%	35	17.2%
	Antiangiogenics	44	19.1%	82	40.2%

Graph 11. Zoledronate cohort; differences in percentages

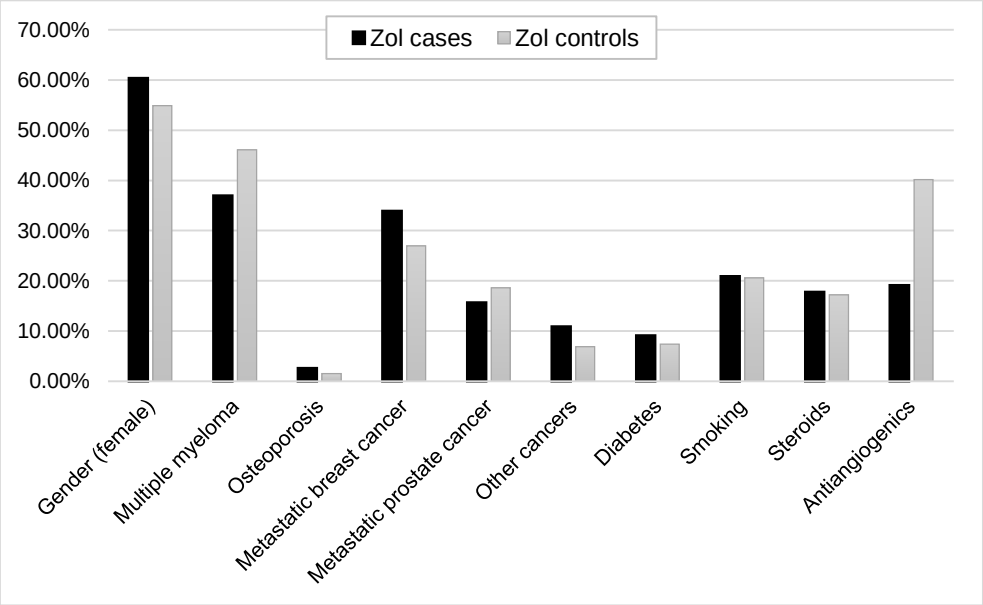


Table 31. Zoledronate all cases and all controls risk factor analysis; random-effects univariable logistic regression

Variable		*N* = 434; case=1, control=0				
		OR	**95% CI**			***p*-value**
Demographics	Age, *decade*	1.46	1.19	to	1.80	<0.001
	Gender (M=0, F=1)	1.16	0.77	to	1.74	0.489
Primary underlying disease		**Multiple myeloma (reference)**				
	Metastatic breast cancer	1.42	0.87	to	2.30	0.158
	Metastatic prostate cancer	0.98	0.54	to	1.75	0.937
	Other cancers	2.22	1.05	to	4.66	0.036
	Osteoporosis	1.55	0.31	to	7.63	0.592
BPs duration, *year*		1.15	1.04	to	1.28	0.010
Systemic factor	Diabetes	1.25	0.60	to	2.61	0.544
	Smoking (no=0, yes/ex=1)	1.05	0.62	to	1.77	0.851
	Steroids	0.85	0.49	to	1.47	0.560
	Antiangiogenics	0.37	0.23	to	0.59	<0.001

* Significant results, $p<0.1$

Table 32. Zoledronate all cases and all controls risk factor analysis; random-effects multivariable logistic regression

Variable		*N* = 434; case=1, control=0				
		Adjusted OR	**95% CI**			***p*-value**
Demographics	Age, *decade*	1.64	1.31	to	2.07	<0.001*
Primary underlying disease		**Multiple myeloma (reference)**				
	Metastatic breast cancer	1.05	0.60	to	1.86	0.864
	Metastatic prostate cancer	0.55	0.29	to	1.06	0.074
	Other cancers	2.24	0.98	to	5.08	0.055
	Osteoporosis	1.20	0.21	to	6.78	0.835
BPs duration, *year*		1.14	1.01	to	1.28	0.029*
Systemic factor	Antiangiogenics	0.37	0.21	to	0.63	<0.001*

* Significant results, $p<0.05$

```
Random-effects logistic regression              Number of obs      =       423
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         1
                                                               avg =      60.4
                                                               max =       359

                                                Wald chi2(7)       =     39.94
Log likelihood  = -247.80784                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
  control0case1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
----------------+-------------------------------------------------------------
     agedecade  |   1.643162   .1929767     4.23   0.000     1.305309    2.068462
                |
disease1op2mm3mbc4mpc5oc |
              1 |   1.201488   1.061016     0.21   0.835     .2128313    6.782705
              3 |   1.051046   .3049317     0.17   0.864     .5952092    1.855984
              4 |   .5498149   .1840336    -1.79   0.074     .2853009    1.059571
              5 |   2.235851   .9361492     1.92   0.055     .9841138    5.079727
                |
durationyearplus1mo |   1.137335   .0672319     2.18   0.029      1.01291    1.277044
         antiang |   .3668259   .1005691    -3.66   0.000     .2143355    .6278064
           _cons |   .8935603   1.630464    -0.06   0.951     .0250006    31.93717
-----------------+-------------------------------------------------------------
        /lnsig2u |   1.560471   1.234561                     -.8592232    3.980165
-----------------+-------------------------------------------------------------
         sigma_u |   2.181986   1.346897                      .6507618    7.316139
             rho |    .591368   .2983339                      .1140452    .9420958
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    40.80 Prob >= chibar2 = 0.000
```

Table 33. Zoledronate exposed type cases and all controls risk factor analysis; random-effects univariable logistic regression

Variable		*N* = 412; case=1, control=0				
		OR	95% CI			*p*-value
Demographics	Age, *decade*	1.47	1.19	to	1.81	<0.001
	Gender (M=0, F=1)	1.05	0.69	to	1.61	0.807
Primary underlying disease		**Multiple myeloma (reference)**				
	Metastatic breast cancer	1.25	0.75	to	2.06	0.391
	Metastatic prostate cancer	0.97	0.53	to	1.76	0.919
	Other cancers	2.17	1.01	to	4.62	0.046
	Osteoporosis	1.68	0.34	to	8.28	0.526
BPs duration, *year*		1.17	1.04	to	1.30	0.007
Systemic factor	Diabetes	1.32	0.63	to	2.78	0.464
	Smoking (no=0, yes/ex=1)	1.05	0.61	to	1.80	0.857
	Steroids	0.97	0.56	to	1.68	0.916
	Antiangiogenics	0.39	0.24	to	0.63	<0.001

* Significant results, $p<0.1$

Table 34. Zoledronate exposed type cases and all controls risk factor analysis; random-effects multivariable logistic regression

Variable		*N* = 412; case=1, control=0				
		Adjusted OR	95% CI			*p*-value
Demographics	Age, *decade*	1.63	1.29	to	2.07	<0.001*
Primary underlying disease		**Multiple myeloma (reference)**				
	Metastatic breast cancer	0.93	0.52	to	1.68	0.820
	Metastatic prostate cancer	0.57	0.29	to	1.11	0.097
	Other cancers	2.15	0.93	to	4.95	0.073
	Osteoporosis	1.33	0.24	to	7.55	0.746
BPs duration, *year*		1.16	1.03	to	1.30	0.017*
Systemic factor	Antiangiogenics	0.38	0.22	to	0.67	0.001*

* Significant results, $p<0.05$

```
Random-effects logistic regression              Number of obs      =       402
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         1
                                                               avg =      57.4
                                                               max =       341

                                                Wald chi2(7)       =     36.76
Log likelihood  = -234.60338                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
      control0case1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------
         agedecade |   1.632859   .1975877     4.05   0.000     1.288093    2.069904
                   |
disease1op2mm3mbc4mpc5oc |
                 1 |   1.332352    1.17886     0.32   0.746     .2352225    7.546733
                 3 |   .9337199    .281048    -0.23   0.820     .5176146    1.684328
                 4 |   .5668481    .193916    -1.66   0.097     .2899177    1.108303
                 5 |   2.147587   .9156893     1.79   0.073     .9311403    4.953208
                   |
durationyearplus1mo |   1.155636   .0699389     2.39   0.017     1.026376    1.301175
           antiang |   .3829983   .1078409    -3.41   0.001     .2205585    .6650738
             _cons |   .8207362   1.489371    -0.11   0.913     .0234179    28.76468
--------------------+---------------------------------------------------------
          /lnsig2u |   1.555411   1.221825                     -.8393212    3.950143
--------------------+---------------------------------------------------------
           sigma_u |   2.176472   1.329634                      .6572698    7.207135
               rho |   .5901446   .2955275                      .1160716    .9404362
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    42.47 Prob >= chibar2 = 0.000
```

Table 35. Zoledronate all cases and super-controls risk factor analysis; random-effects univariable logistic regression

Variable		*N* = 318; case=1, control=0				
		OR	95% CI			*p*-value
Demographics	Age, *decade*	1.61	1.22	to	2.11	0.001
	Gender (M=0, F=1)	1.04	0.62	to	1.75	0.879
Primary underlying disease		**Multiple myeloma (reference)**				
	Metastatic breast cancer	1.70	0.93	to	3.09	0.082
	Metastatic prostate cancer	1.73	0.78	to	3.81	0.176
	Other cancers	3.91	1.26	to	12.09	0.018
	Osteoporosis	2.50	0.26	to	23.70	0.424
BPs duration, *year*		0.88	0.78	to	0.98	0.026
Systemic factor	Diabetes	1.36	0.52	to	3.58	0.534
	Smoking (no=0, yes/ex=1)	1.15	0.60	to	2.19	0.672
	Steroids	0.75	0.39	to	1.46	0.405
	Antiangiogenics	0.28	0.16	to	0.49	<0.001

* Significant results, $p<0.1$

Table 36. Zoledronate all cases and super-controls risk factor analysis; random-effects multivariable logistic regression

Variable		*N* = 318; case=1, control=0				
		Adjusted OR	95% CI			*p*-value
Demographics	Age, *decade*	1.72	1.26	to	2.34	0.001*
Primary underlying disease		**Multiple myeloma (reference)**				
	Metastatic breast cancer	1.46	0.71	to	3.02	0.305
	Metastatic prostate cancer	0.76	0.31	to	1.83	0.539
	Other cancers	3.87	1.16	to	12.84	0.027*
	Osteoporosis	1.22	0.12	to	12.76	0.871
BPs duration, *year*		0.85	0.75	to	0.96	0.012*
Systemic factor	Antiangiogenics	0.27	0.14	to	0.53	<0.001*

* Significant results, $p<0.05$

```
Random-effects logistic regression              Number of obs      =       308
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         1
                                                               avg =      44.0
                                                               max =       245

                                                Wald chi2(7)       =     36.41
Log likelihood  = -155.75418                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
      control0case1 |      OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------
        agedecade |   1.720325   .2717417     3.43   0.001     1.262278    2.344584
                  |
disease1op2mm3mbc4mpc5oc |
                1 |   1.215054    1.45772     0.16   0.871     .1157163     12.7584
                3 |   1.461376   .5402008     1.03   0.305     .7081309    3.015855
                4 |    .757711   .3418003    -0.62   0.539     .3129898    1.834328
                5 |   3.867291   2.368035     2.21   0.027     1.164651    12.84156
                  |
durationyearplus1mo |   .8476574   .0556472    -2.52   0.012     .7453161    .9640515
          antiang |   .2730945   .0912799    -3.88   0.000      .141842    .5258006
            _cons |   .7630063   1.194809    -0.17   0.863     .0354497    16.42267
--------------------+---------------------------------------------------------
         /lnsig2u |   .5753094   1.385041                     -2.139322     3.28994
--------------------+---------------------------------------------------------
          sigma_u |   1.333297   .9233355                      .3431249    5.180855
              rho |   .3507969   .3154271                      .0345506     .890815
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    10.95 Prob >= chibar2 = 0.000
```

Table 37. Alendronate cohort; descriptive statistics

		Cases *N* = 109		Controls *N* = 31	
		n	**%**	***n***	**%**
Age, *decade*	Mean, median	7.2, 7.2		6.8, 7.0	
	SD	1.0		0.9	
	Range	3.7 to 8.9		4.7 to 8.3	
Gender	Female	98	89.9%	29	93.5%
	Male	11	10.1%	2	6.5%
Primary underlying disease	Multiple myeloma	2	1.8%	0	0.0%
	Osteoporosis	104	95.4%	28	90.3%
	Metastatic breast cancer	3	2.8%	3	9.7%
	Metastatic prostate cancer	0	0.0%	0	0.0%
	Other cancers	0	0.0%	0	0.0%
BPs duration, *year*	Mean, median	6.1, 5.8		4.9, 3.7	
	SD	3.0		3.8	
	Range	1.0 to 15.1		1.1 to 16.0	
Systemic factor	Diabetes	12	11.0%	2	6.5%
	Smoking	26	23.9%	3	9.7%
	Steroids	31	28.4%	1	3.2%
	Antiangiogenics	6	5.5%	0	0.0%

Graph 12. Alendronate cohort; differences in percentages

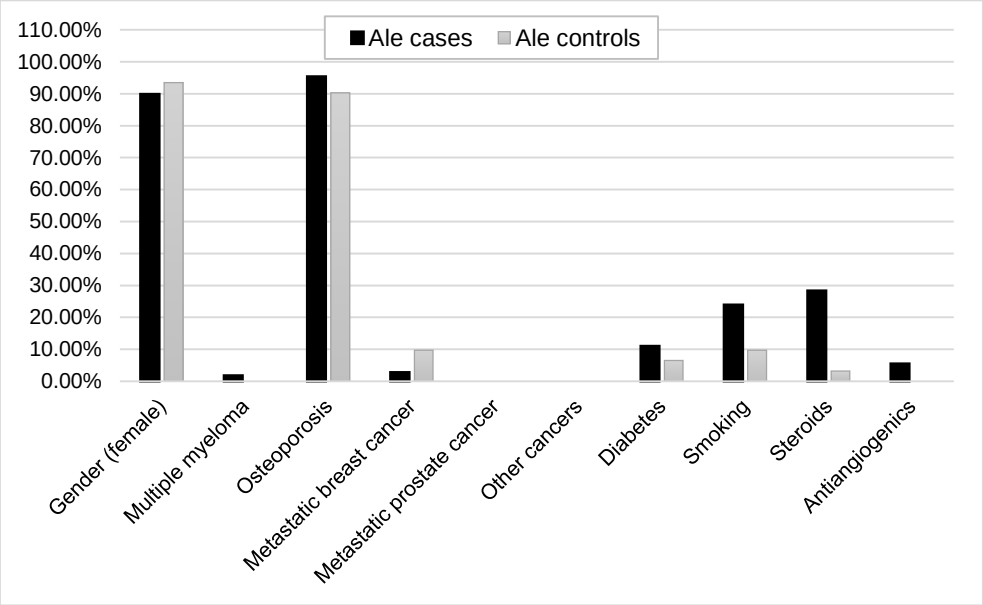


Table 38. Alendronate all cases and all controls risk factor analysis; random-effects univariable logistic regression

Variable		*N* = 140; case=1, control=0				
		OR	**95% CI**			***p*-value**
Demographics	Age, *decade*	1.52	0.95	to	2.44	0.078
	Gender (M=0, F=1)	0.98	0.18	to	5.42	0.982
BPs duration, *year*		1.14	0.98	to	1.32	0.087
Systemic factor	Diabetes	1.64	0.32	to	8.27	0.552
	Smoking (no=0, yes/ex=1)	2.38	0.62	to	9.06	0.204
	Steroids	10.59	1.35	to	83.15	0.025

* Significant results, $p<0.1$

Table 39. Alendronate all cases and all controls risk factor analysis; random-effects multivariable logistic regression

Variable		*N* = 140; case=1, control=0				
		Adjusted OR	**95% CI**			***p*-value**
Demographics	Age, *decade*	1.44	0.82	to	2.52	0.201
BPs duration, *year*		1.12	0.96	to	1.31	0.137
Systemic factor	Steroids	9.61	1.19	to	77.49	0.034*

* Significant results, $p<0.05$

```
Random-effects logistic regression              Number of obs      =       130
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         5
                                                               avg =      18.6
                                                               max =        77

                                                Wald chi2(3)       =      8.74
Log likelihood  = -60.294216                    Prob > chi2        =    0.0330

------------------------------------------------------------------------------
      control0case1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------------
          agedecade |    1.44071   .4114249     1.28   0.201      .823186    2.521477
durationyearplus1mo |   1.122129   .0870279     1.49   0.137     .9638886    1.306347
            steroid |   9.608944   10.23406     2.12   0.034     1.191512    77.49127
              _cons |   .1700376   .3780319    -0.80   0.425     .0021784    13.27266
--------------------+---------------------------------------------------------------
           /lnsig2u |  -.9931067   1.992145                     -4.897639    2.911425
--------------------+---------------------------------------------------------------
            sigma_u |   .6086248   .6062343                      .0863955    4.287538
                rho |   .1012007   .1812037                      .0022637    .8482032
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.78 Prob >= chibar2 = 0.188
```

Table 40. Alendronate exposed type cases and all controls risk factor analysis; random-effects univariable logistic regression

Variable		*N* = 120; case=1, control=0				
		OR	**95% CI**			***p*-value**
Demographics	Age, *decade*	1.59	0.97	to	2.59	0.064
	Gender (M=0, F=1)	1.09	0.18	to	6.63	0.926
BPs duration, *year*		1.14	0.98	to	1.33	0.086
Systemic factor	Diabetes	1.97	0.38	to	10.14	0.419
	Smoking (no=0, yes/ex=1)	2.17	0.55	to	8.54	0.266
	Steroids	11.94	1.51	to	94.45	0.019

* Significant results, $p<0.1$

Table 41. Alendronate exposed type cases and all controls risk factor analysis; random-effects multivariable logistic regression

Variable		*N* = 120; case=1, control=0				
		Adjusted OR	**95% CI**			***p*-value**
Demographics	Age, *decade*	1.65	0.91	to	3.00	0.099
BPs duration, *year*		1.12	0.96	to	1.31	0.151
Systemic factor	Steroids	12.07	1.45	to	100.22	0.021*

* Significant results, $p<0.05$

```
Random-effects logistic regression              Number of obs      =       111
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         4
                                                               avg =      15.9
                                                               max =        65

                                                Wald chi2(3)       =      9.93
Log likelihood  = -53.626716                    Prob > chi2        =    0.0191

------------------------------------------------------------------------------
      control0case1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------
          agedecade |   1.652229   .5028146     1.65   0.099     .9099729    2.999937
durationyearplus1mo |   1.119731   .0881522     1.44   0.151     .9596259    1.306549
            steroid |   12.07205   13.03632     2.31   0.021     1.454082    100.2244
              _cons |   .0522791   .1235148    -1.25   0.212     .0005097    5.362669
--------------------+---------------------------------------------------------
           /lnsig2u |   -.871987   1.781273                     -4.363217    2.619243
--------------------+---------------------------------------------------------
            sigma_u |   .6466219    .575905                      .1128598    3.704771
                rho |   .1127619   .1782104                      .0038568    .8066512
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     1.08 Prob >= chibar2 = 0.150
```

Table 42. Summary of risk factor analysis

	Overall cohort *N* = 669	**Zoledronate all cases and all controls *N* = 434**	**Zoledronate exposed type cases and all controls *N* = 412**	**Zoledronate all cases and super-controls *N* = 318**
Significant risk factors	- Age - BPs duration	- Age - BPs duration	- Age - BPs duration	- Age - Other cancers
Significant protective factors	- Antiangiogenics	- Antiangiogenics	- Antiangiogenics	- Antiangiogenics (with BPs duration adjusted)

	Alendronate all cases and all controls *N* = 140	**Alendronate exposed type cases and all controls *N* = 120**
Significant risk factors	- Steroids	- Steroids
Significant protective factors	/	/

5.3.3 Post-hoc analysis

In the overall and zoledronate cohorts, age, BPs duration, other cancers and antiangiogenics were found statistically significant. It would be useful to study the relationship between these four factors, two factors at a time, and their results can be found below.

Meanwhile, as there was only one significant factor identified in the alendronate cohorts, i.e. use of steroids, post-hoc analysis to study the interrelationship between factors was not indicated.

5.3.3.1 Relationships between age and BPs duration, other cancers, and antiangiogenics

The relationship between age and BPs duration is first presented in Graph 13. The vertical axis represents BPs duration, in number of years, while the horizontal axis was age in decade. Each dot represents an individual, including both cases and controls. The trend line illustrates that when age increases, BPs duration is also found to increase.

It was also found that age was statistically significantly associated with BPs duration (p=0.003) (Table 43). An increase in age by 10 years was associated with an increase in BPs duration by 0.34 years (95% CI 0.11 to 0.57 years).

Concerning the relationship between age and underlying diseases, descriptive statistics reported that other cancers patients, mainly kidney or lung cancers, were the youngest (median: 65 years old), followed by metastatic breast cancer patients, with a median age of 66 years old. Multiple myeloma patients had a median age of 69 years old, while metastatic prostate cancer patients were the oldest (median: 73 years old) (Table 44).

There was also a statistically significant association between age and other cancers (Table 44). Patients diagnosed with other cancers, were found statistically significantly younger than those with multiple myeloma (p=0.004) and metastatic prostate cancer (p<0.001). On average, other cancers patients were by nearly five years younger than the multiple myeloma patients (estimated coefficient 0.49 decades, 95% CI 0.16 to 0.82 decades), and by nearly 10 years younger than the metastatic prostate cancer patients (estimated coefficient 0.96 decades, 95% CI 0.58 to 1.33 decades). Although not statistically significant, other cancers patients were also found slightly younger than the metastatic breast cancer patients (estimated coefficient 0.17 decades, 95% CI -0.17 to 0.51 decades, p=0.326).

Lastly, those who were on antiangiogenics were found younger than the non-users, by approximately two years on average (Table 45). However, this was not found statistically significant (estimated coefficient -0.17 decades, 95% CI -0.36 to 0.02 decades, p=0.076).

5.3.3.2 Relationships between BPs duration and other cancers, and antiangiogenics

As reported above, BPs duration was longer amongst older patients. However, it was found shorter amongst individuals with other cancers.

Descriptive statistics showed that metastatic prostate cancer patients had the shortest BPs duration (median: 1.3 years). This was followed by patients with other metastatic cancers, whose BPs duration median was 1.4 years. Multiple myeloma patients had a median of 1.8 years, while metastatic breast cancer patients were on the longest length of BPs (median: 2.1 years) (Table 46).

Inferential statistics also calculated that, on average, BPs duration amongst the other cancers patients was by 0.97 years shorter than in the metastatic breast cancer patients (95% CI 0.21 to 1.72 years, p=0.012), and by 0.60 years shorter than in the multiple myeloma patients, although not statistically significant (95% CI -0.13 to 1.33 years, p=0.107) (Table 46). However, metastatic prostate cancer patients had an even shorter BPs duration than other cancers patients, although it was also not statistically significant (estimated coefficient -0.33 years, 95% CI -1.16 to 0.50 years, p=0.439).

The relationship between BPs duration and antiangiogenics, the only protective factor identified, was also investigated. Those prescribed with antiangiogenics (median: 1.8 years) had a shorter BPs duration than the non-users (median: 2.4 years). The difference was also found statistically significant (estimated coefficient -1.04 years, 95% CI -1.59 to -0.48 years, p<0.001) (Table 47).

5.3.3.3 Relationship between other cancers and antiangiogenics

To recapitulate, for other cancers patients, they were found statistically significantly younger than the multiple myeloma and metastatic prostate cancer patients. They were also on significantly shorter BPs than the metastatic breast cancer patients.

As for patients managed with antiangiogenics, compared with the non-users, their BPs duration was statistically significantly shorter. However, there was no significant difference in age between the two groups.

Lastly, it was found that metastatic breast cancer patients had the smallest proportion of individuals prescribed with concomitant antiangiogenics (10.8%) (Table 48). This was followed by metastatic prostate cancer patients (14.7%) and other cancers patients (22.0%). Lastly, multiple myeloma patients had the largest proportion of individuals prescribed with antiangiogenics (55.2%).

It was also found that the proportion of patients prescribed with antiangiogenics was statistically significantly higher amongst the multiple myeloma patients than in the other cancers patients (OR=4.11, 95% CI 1.86 to 9.11, $p<0.001$). But the differences were not found statistically significant when other cancers patients were compared with metastatic breast cancer or prostate cancer patients (metastatic breast cancer: OR=0.43, 95% CI 0.17 to 1.07, $p=0.070$; metastatic prostate cancer: OR=0.62, 95% CI 0.23 to 1.65, $p=0.338$).

5.3.3.4 Summary

In summary, older age, a risk factor, was associated with longer BPs duration, another risk factor (Table 49). However, patients with other cancers, also a risk factor, were found younger and were on shorter BPs therapy. On the other hand, patients on antiangiogenics, the only protective factor, were also on shorter BPs and had less individuals diagnosed with other cancers. Lastly, age and use of antiangiogenics were not found to be related to each other.

Graph 13. Relationship between age and BPs duration; descriptive statistics

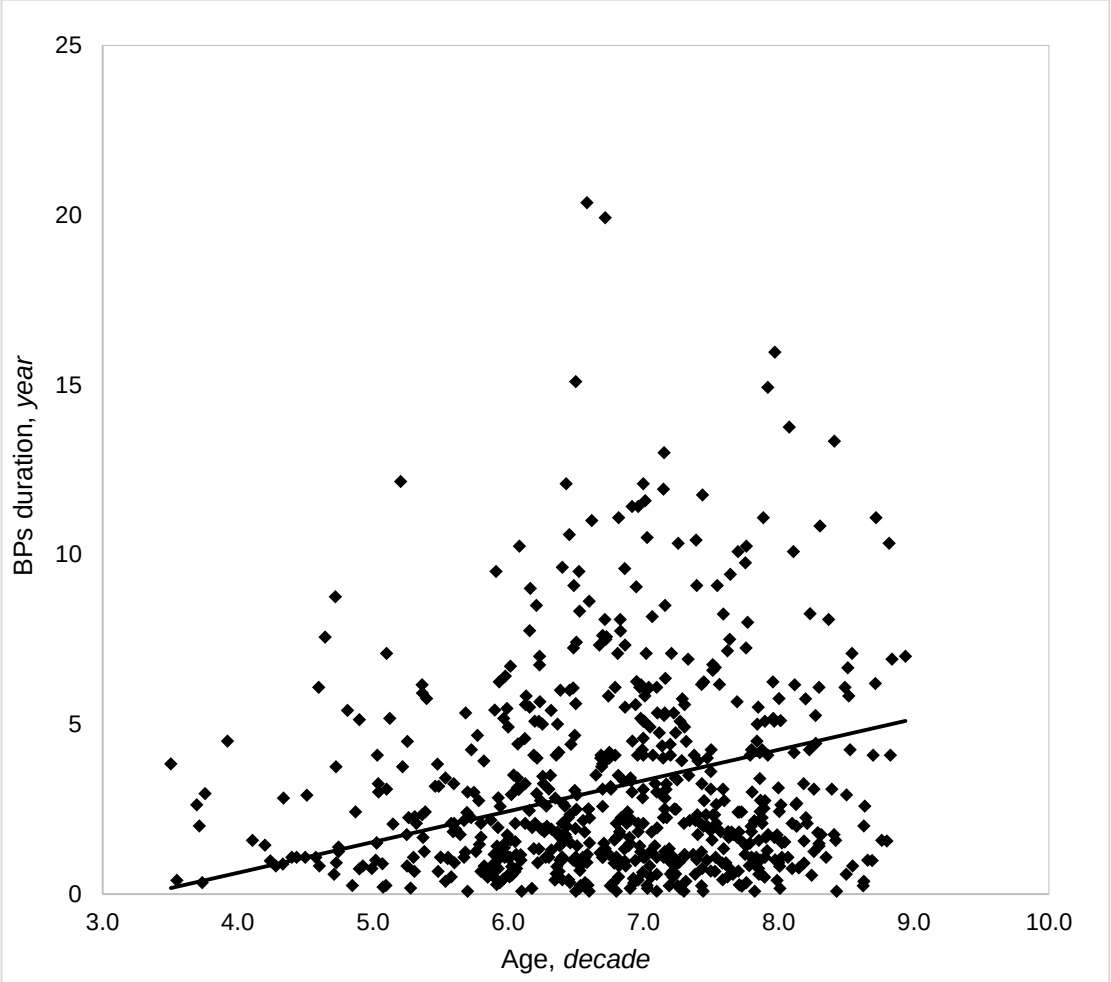


Table 43. Relationship between age and BPs duration; random-effects univariable linear regression

	Estimated coefficient	95% CI	*p*-value
Age, *decade*	0.34	0.11 to 0.57	0.003*

* Significant results, $p<0.05$

```
Random-effects ML regression                    Number of obs      =       643
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         8
                                                               avg =      91.9
                                                               max =       500

                                                LR chi2(1)         =      8.59
Log likelihood  =  -1617.063                    Prob > chi2        =    0.0034

------------------------------------------------------------------------------
durationyearplus1mo |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------
          agedecade |   .3408038   .1159028     2.94   0.003     .1136384    .5679691
              _cons |   1.370327    .878688     1.56   0.119    -.3518697    3.092524
--------------------+---------------------------------------------------------
           /sigma_u |   .6037081   .3382039                      .2013606    1.810004
           /sigma_e |   2.976109    .083522                       2.81683    3.144395
                rho |   .0395224   .0427189                      .0030963    .2191381
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    4.25 Prob>=chibar2 = 0.020
```

Table 44. Relationship between age and other cancers

	Age, *decade*	Estimated coefficient	95% CI			*p*-value
Other cancers ***N* = 41**	Mean; median 6.3; 6.5 SD; range 1.1; 3.5 to 8.5	**(reference)**				
Multiple myeloma ***N* = 209**	Mean; median 6.8; 6.9 SD; range 1.0; 3.7 to 8.8	0.49	0.16	to	0.82	0.004*
MBC ***N* = 152**	Mean; median 6.4; 6.6 SD; range 1.1; 3.5 to 8.7	0.17	-0.17	to	0.51	0.326
MPC ***N* = 75**	Mean; median 7.2; 7.3 SD; range 0.7; 4.7 to 8.6	0.96	0.58	to	1.33	<0.001*

* Significant results, *p*<0.05

```
Random-effects ML regression                    Number of obs      =       477
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         1
                                                               avg =      68.1
                                                               max =       400

                                                LR chi2(3)         =     38.68
Log likelihood  = -672.86581                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
             agedecade |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-----------------------+------------------------------------------------------------------
disease1oc2mm3mbc4mpc |
                    2  |   .4928414      .1692     2.91   0.004     .1612156    .8244672
                    3  |   .1718212   .1750012     0.98   0.326    -.1711749    .5148173
                    4  |   .9581562   .1921602     4.99   0.000     .5815291    1.334783
                       |
                 _cons |   6.196751    .183421    33.78   0.000     5.837252    6.556249
-----------------------+------------------------------------------------------------------
              /sigma_u |   .1483004   .1216415                      .0297136    .7401662
              /sigma_e |   .9879315   .0321644                      .9268597    1.053027
                   rho |   .0220371   .0354748                      .0004246    .2449305
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    1.07 Prob>=chibar2 = 0.150
```

Table 45. Relationship between age and antiangiogenics

	Age, *decade*	Estimated coefficient	95% CI			*p*-value
Non-antiangiogenics users *N* = 518	Mean; median 6.8; 6.9 SD; range 1.0; 3.5 to 8.9	**(reference)**				
Antiangiogenics users *N* = 150	Mean; median 6.6; 6.7 SD; range 1.1; 3.5 to 8.7	-0.17	-0.36	to	0.02	0.076

* Significant results, *p*<0.05

```
Random-effects ML regression                    Number of obs      =       668
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         8
                                                               avg =      95.4
                                                               max =       505

                                                LR chi2(1)         =      3.14
Log likelihood  =  -968.9758                    Prob > chi2        =    0.0765

------------------------------------------------------------------------------
   agedecade |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     antiang |  -.1709936    .096428    -1.77   0.076    -.3599891    .0180019
       _cons |   6.922934   .1185134    58.41   0.000     6.690652    7.155216
-------------+----------------------------------------------------------------
    /sigma_u |   .2069043   .1169383                      .0683419    .6264006
    /sigma_e |   1.026627   .0282773                       .972674    1.083573
         rho |   .0390321   .0425878                      .0029784    .2194926
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    1.25 Prob>=chibar2 = 0.132
```

Table 46. Relationship between BPs duration and other cancers

	BPs duration, *year*	Estimated coefficient	95% CI			*p*-value
Other cancers ***N* = 41**	Mean; median 1.9; 1.4 SD; range 1.5; 0.2 to 6.6	**(reference)**				
Multiple myeloma ***N* = 201**	Mean; median 2.5; 1.8 SD; range 2.3; 0.1 to 13.0	0.60	-0.13	to	1.33	0.107
MBC ***N* = 148**	Mean; median 2.9; 2.1 SD; range 2.5; 0.1 to 12.1	0.97	0.21	to	1.72	0.012*
MPC ***N* = 75**	Mean; median 1.6; 1.3 SD; range 1.3; 0.1 to 6.4	-0.33	-1.16	to	0.50	0.439

* Significant results, *p*<0.05

```
Random-effects ML regression                    Number of obs      =       465
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         1
                                                               avg =      66.4
                                                               max =       397

                                                LR chi2(3)         =     19.71
Log likelihood  = -1022.2313                    Prob > chi2        =    0.0002

------------------------------------------------------------------------------
  durationyearplus1mo |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
----------------------+-------------------------------------------------------
disease1oc2mm3mbc4mpc |
                    2 |   .6021902   .3735855     1.61   0.107    -.1300239    1.334404
                    3 |   .9659151   .3847516     2.51   0.012     .2118159    1.720014
                    4 |  -.3273354   .4234272    -0.77   0.439    -1.157237    .5025666
                      |
                _cons |   1.893618   .3404712     5.56   0.000     1.226307     2.56093
----------------------+-------------------------------------------------------
             /sigma_u |          0   .1058981                             .            .
             /sigma_e |   2.180182   .0714826                      2.044485    2.324885
                  rho |          0  (omitted)
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.00 Prob>=chibar2 = 1.000
```

Table 47. Relationship between BPs duration and antiangiogenics

	BPs duration, *year*	Estimated coefficient	95% CI			*p*-value
Non-antiangiogenics users *N* = 497	Mean; median 3.5; 2.4 SD; range 3.2; 0.1 to 20.4	**(reference)**				
Antiangiogenics users *N* = 146	Mean; median 2.3; 1.8 SD; range 2.2; 0.2 to 13.0	-1.04	-1.59	to	-0.48	<0.001*

* Significant results, *p*<0.05

```
Random-effects ML regression                    Number of obs      =       643
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         8
                                                               avg =      91.9
                                                               max =       500

                                                LR chi2(1)         =     13.21
Log likelihood  = -1614.7574                    Prob > chi2        =    0.0003

------------------------------------------------------------------------------
durationyearplus1mo |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------
           antiang |  -1.035916   .2839912    -3.65   0.000    -1.592529   -.4793037
             _cons |   3.874373    .336901    11.50   0.000     3.214059    4.534687
--------------------+---------------------------------------------------------
          /sigma_u |   .5901826   .3642284                      .1760643    1.978342
          /sigma_e |   2.965774    .083326                      2.806874    3.133671
               rho |   .0380917   .0454305                      .0021962     .242578
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    2.50 Prob>=chibar2 = 0.057
```

Table 48. Relationship between other cancers and antiangiogenics

	Use of antiangiogenics	OR	95% CI			*p*-value
Other cancers *N* = 41	*N* = 9; 22.0%		**(reference)**			
Multiple myeloma *N* = 201	*N* = 111; 55.2%	4.11	1.86	to	9.11	<0.001*
MBC *N* = 148	*N* = 16; 10.8%	0.43	0.17	to	1.07	0.070
MPC *N* = 75	*N* = 11; 14.7%	0.62	0.23	to	1.65	0.338

* Significant results, $p<0.05$

```
Random-effects logistic regression              Number of obs      =       478
Group variable: country                         Number of groups   =         7

Random effects u_i ~ Gaussian                   Obs per group: min =         1
                                                               avg =      68.3
                                                               max =       400

                                                Wald chi2(3)       =     76.12
Log likelihood  =  -249.1721                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
             antiang |          OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
---------------------+--------------------------------------------------------------
disease1oc2mm3mbc4mpc |
                   2 |   4.111549   1.669348     3.48   0.000     1.855262    9.111829
                   3 |   .4319143   .2003892    -1.81   0.070     .1739712    1.072303
                   4 |   .6190657   .3095763    -0.96   0.338     .2323145     1.64967
                     |
               _cons |   .2442107   .1114292    -3.09   0.002     .0998564    .5972462
---------------------+--------------------------------------------------------------
            /lnsig2u |  -2.913035   2.691662                     -8.188595    2.362525
---------------------+--------------------------------------------------------------
             sigma_u |   .2330465   .3136411                      .0166675    3.258486
                 rho |   .0162404   .0430036                      .0000844    .7634481
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.07 Prob >= chibar2 = 0.392
```

Table 49. Summary of post-hoc analysis

		Significant risk factor			Significant protective factor
		Age	**BPs duration**	**Other cancers**	**Anti-angiogenics**
Significant risk factor	**Age**	/	Significant; ↑ BPs duration ~ ↑ age	Significant; other cancers ~ ↓ age	Not significant
	BPs duration	Significant; ↑ age ~ ↑ BPs duration	/	Significant; other cancers ~ ↓ BPs duration	Significant; anti-angiogenics ~ ↓ BPs duration
	Other cancers	Significant; ↑ age ~ ↓ other cancers	Significant; ↑ BPs duration ~ ↓ other cancers	/	Significant; anti-angiogenics ~ ↓ other cancers
Significant protective factor	**Anti-angiogenics**	Not significant	Significant; ↑ BPs duration ~ ↓ anti-angiogenics	Significant; other cancer ~ ↓ anti-angiogenics	/

5.4 Discussion

5.4.1 Main findings and comparison with previous studies

5.4.1.1 Cohort stratification

5.4.1.1.1 Overall, zoledronate and alendronate cohorts

Results from the overall cohort were found similar to the three zoledronate cohorts. This may be explained by the fact that over 60% of the entire cohort was managed with zoledronate, hence similar significant factors were found.

Amongst the three zoledronate cohorts, their results were similar. All three identified age and antiangiogenics as significant factors. BPs duration was also found a significant risk factor for ONJ development in two of the cohorts, except in the one with super-controls when BPs duration was adjusted.

Results from the two alendronate cohorts had also been consistent, as both identified the use of steroids as the only significant risk factor.

So, the significant factors identified in the zoledronate and the alendronate cohorts were found different. This may be explained by findings from earlier parts of the current study. In 5.3.1.1, it was found that the zoledronate and alendronate users, including both cases and controls, were largely phenotypically different, with respect to age, gender, underlying diseases and BPs duration. In Chapter 4, it was reported that both time to onset and follow-up time were shorter amongst those on zoledronate than the alendronate patients. Chapter 3 also reported that their cases had different proportion of individuals presented with different ONJ types. Therefore, it is possible that ONJ related to different types of BPs may have different pathophysiology mechanisms.

This also helps prove the importance of cohort stratification. If, similar to most previous studies, there was only one analysis for all participants on different types of BPs, steroids as a risk factor amongst the alendronate patients could never be identified. Also, its results would be biased and can represent its largest group only, such as the zoledronate patients in the case of GENVABO.

Nevertheless, cohort stratification in ONJ risk factor analysis remains new; the alendronate cohort was also small, in particular the alendronate-exposed controls. Therefore, the current findings remain preliminary.

5.4.1.1.2 Exposed type case cohorts

Concerning the exposed type cases cohorts, both the zoledronate and alendronate cohorts shared the same results with their respective all cases cohorts. For zoledronate, both had age and BPs duration as risk factors and antiangiogenics as protective factor, while the use of steroids was identified as a risk factor for both the alendronate all case and exposed type cases cohorts.

This further supported findings from Chapter 3 that the exposed and non-exposed types were similar. In other words, the inclusion of the non-exposed cases into the all cases cohorts did not change the statistical results. However, this may be explained by the fact that the majority of the cases were of the exposed type and the removal of approximately 20 non-exposed cases from each cohort, i.e. 5.1% and 14.3% respectively for the zoledronate and alendronate case cohorts, was not significant enough to change the final results. Nonetheless, this was a first attempt using an alternative approach to investigate whether or not the two ONJ types are similar.

5.4.1.1.3 Super-controls cohort

As reported in 5.3.1.2, zoledronate super-controls, i.e. controls who had been reviewed for 2.1 years or longer, and controls reviewed for less than 2.1 years were found largely phenotypically similar. This explained the largely similar results shared by the zoledronate super-controls cohort and the other two zoledronate cohorts, i.e. age as a significant risk factor and antiangiogenics as a significant protective factor were reported in all three cohorts.

However, the main difference was with BPs duration. BPs duration of super-controls was by approximately two years longer than the other controls. Therefore, in the all controls cohorts, i.e. all controls managed with zoledronate with different follow-up time, BPs duration was found a significant risk factor for ONJ. When there were only super-controls, BPs duration was adjusted, or, it became a significant protective factor. An increase in BPs duration by a year was associated with a decrease in the odds of having ONJ by about 15% (95% CI 0.75 to 0.96, p=0.012).

In fact, the all controls cohorts in the current study represented most previous ONJ risk factor studies, while the super-controls cohort illustrated the importance of control validation.

As explained in Chapter 4, the criteria in control screening had been inconsistent in most previous studies. Also, none performed screening using the actual follow-up time. Therefore, these controls may have been “under-reviewed”, i.e. the opposite of super-controls, and have a high risk of being a “false-control”. These controls are also more likely to have a shorter BPs duration (Palaska et al., 2009). As a result, BPs duration was often found

longer amongst the ONJ cases and was also more likely to be reported a risk factor, which was also the case in the two zoledronate all control cohorts in the current study as predicted (Thumbigere-Math et al., 2012; Sedghizadeh et al., 2013; Tsao et al., 2013).

Meanwhile, once with control validation, BPs duration was no longer a significant risk factor. This suggested that BPs duration, a traditional risk factor for ONJ, may not be as strong as researchers think; and it may have been resulted from sampling bias in most previous studies. Further discussion on BPs duration will continue in 5.4.1.2.1.

Another risk factor, other cancers, not found significant in the other two zoledronate cohorts, became a significant risk factor in the super-controls cohort. This may be because, as reported in 5.3.1.2, there were fewer other cancers patients amongst the super-controls than the controls reviewed for less than 2.1 years, although the difference was not statistically significant. Although other cancers was significant as a risk factor (p=0.027), it had a relatively large standard error (2.37), and its 95% CI was also wide (1.16 to 12.84). Therefore, this finding remains preliminary.

In conclusion, analysing with super-controls should help minimise the risk of potential “false-controls” and thus yielding more robust risk factor results.

5.4.1.2 Significant risk factors

5.4.1.2.1 BPs duration

BPs duration, a traditional risk factor for ONJ, was also found a significant risk factor in three out of six cohorts in the current study. An increase in a year of BPs duration was associated with a 13 to 16% increase in the odds of having ONJ. This applied to the overall cohort, the zoledronate all cases and all controls cohort, and the zoledronate exposed type cases and all controls cohort.

This had also been supported by three previous multivariable studies, including a large study reporting a 2.5-year difference in BPs duration between 18 cases and 558 controls (Thumbigere-Math et al., 2012). A more recent study also reported a difference of 2.6 years in BPs duration between 69 cases and 84 controls (Sedghizadeh et al., 2013). This smaller study, with 22 cases and 41 controls, also found BPs duration by 1.2 years longer amongst the cases, which was closer to the results of the current study in which a difference of 1.1 years was reported in the overall cohort (Tsao et al., 2013). Of note, the differences in BPs duration were all statistically significant in these three studies.

Although BPs duration was also found longer amongst the alendronate cases than the controls, unlike in the overall and the zoledronate cohorts, it was not found statistically significant at the multivariable level in the two alendronate cohorts in the current study. This may be because the length of BPs therapy was found statistically significantly different between the zoledronate and the alendronate users, as reported in the pre-risk factor analysis in 5.3.1.1, as well as different case-control ratio in the alendronate and the zoledronate cohorts.

There were also two previous multivariable studies reporting no statistically significant difference in BPs duration between ONJ cases and controls. They were Thumbigere-Math et al., 2013 and Katz et al., 2011, which studied 25 cases and 48 controls, and 12 cases and 66 controls respectively.

On the other hand, with regard to the post-hoc analysis in the current study, BPs duration was found to correlate with age, another risk factor. This applied to the overall cohort, the zoledronate all cases and all controls cohort, and the zoledronate exposed type cases and all controls cohort. However, interestingly, in the super-controls cohort, regardless of duration of BPs therapy being significantly shorter amongst the cases than the controls, the case patients remained significantly older.

In summary, it remains unclear whether BPs duration is a true ONJ risk factor, or a confounding factor, or, as discussed in 5.4.1.1.3, it may have been overestimated in some cohorts and in some previous studies due to sampling bias involving the recruitment of the inadequately reviewed “false-controls”.

5.4.1.2.2 Age

Age, another risk factor identified in the current study, was significant in the overall and all three zoledronate cohorts. On average, the cases were by approximately three years older than the controls, and an increase in age by 10 years was associated with a 56 to 72% increase in the odds of having ONJ.

Probably because there was a significant difference in age between the zoledronate and the alendronate users, as reported in the pre-risk factor analysis, it was not found a significant risk factor in the two alendronate cohorts, although the alendronate cases were also found older than the controls.

Age as an ONJ risk factor has also been supported by two previous multivariable studies. Sedghizadeh et al., 2013 reported that their 69 cases were by six years older than the 84 controls, while a larger study, Vahtsevanos et al., 2009, observed a 3.6 years age difference between the 80 cases and 1,541 controls, which was close to the results of the current study. Of note, both differences were statistically significant.

However, the mechanism of how age relates to ONJ development remains largely unknown as this has never been formally studied. Traditionally, age was not perceived as an ONJ risk factor. Therefore, it was often adjusted in the final multivariable model with its statistical results unreported (Thumbigere-Math et al., 2013; Tsao et al., 2013), or, matched between the cases and controls in some other studies (Kyrgidis et al., 2008; Wessel et al., 2008; Tsao et al., 2013).

In the post-hoc analysis in the current study, it was found that age correlated with BPs duration, which may explain, probably due to its relationship with BPs, age became a significant risk factor. However, in the zoledronate super-controls cohort, when BPs duration was no longer a risk factor, interestingly, the results for age remained the same. This important finding suggested that, age was unlikely to be a confounder and may have direct association with ONJ.

There were also previous studies reporting that cases were younger than the controls, but the difference was not statistically significant (Kyrgidis et al., 2008; Katz et al., 2011; Thumbigere-Math et al., 2012).

5.4.1.2.3 Steroids

In the two alendronate cohorts, use of steroids, an immunosuppressant, was found a significant risk factor. This was also supported by Thumbigere-Math et al., 2012, a study of 18 cases and 558 controls who were on zoledronate and/or pamidronate.

However, the same did not apply to the zoledronate cohorts or the overall cohort in the current study. Also, even in the two alendronate cohorts, this finding needs to be interpreted with caution. Amongst the 109 alendronate cases, there were 31 patients on steroids. In contrast, there was only one steroid user amongst the 31 alendronate-exposed controls recruited. As a result, steroids, though statistically significant, showed a wide 95% CI for the OR (1.19 to 77.49), as well as a large standard error (10.23).

Therefore, as summarised in a recent review, data for steroids have been considered occasionally positive but remained not well-proven (Campisi et al., 2014).

5.4.1.2.4 Other cancers

Other cancers, mainly kidney or lung cancers, was also found a significant risk factor in the zoledronate super-controls cohort only in the current study. There were a small number of individuals presented with other cancers, i.e. 27 out of 393 cases versus 14 out of 276 controls. As a result, its OR also showed a wide 95% CI (1.16 to 12.84) and a large standard error (2.37).

In the literature, other cancers had never been reported a risk factor for ONJ (Wessel et al., 2008; Thumbigere-Math et al., 2012, 2013). Hence the reason why these patients are presented with a higher risk of ONJ remains unknown.

In the current post-hoc analysis, other cancers did not appear to associate positively with any other risk factors. First, instead of being older, these patients were found younger, and were also on shorter BPs, than the multiple myeloma, metastatic prostate, and metastatic breast cancer patients. This suggested that other cancers *per se* may have an effect towards ONJ, but its role as a potential risk factor remains largely preliminary.

5.4.1.3 Significant protective factor

5.4.1.3.1 Antiangiogenics

From the mainstream understanding of ONJ development, antiangiogenics has long been considered a robust risk factor (Campisi et al., 2014). However, there were also studies observing no differences in the proportion of antiangiogenics users between the cases and controls (Thumbigere-Math et al., 2012; Tsao et al., 2013). Surprisingly, in the current study, the use of antiangiogenics was found a protective factor in the overall and the three zoledronate cohorts. Of note, due to statistical consideration, antiangiogenics was excluded from analysis in the alendronate cohorts as none of their controls were managed with concomitant antiangiogenics.

Interestingly, the current post-hoc analysis found that those on antiangiogenics had a much shorter BPs duration than the non-users. There was also a smaller proportion of other cancers patients prescribed with antiangiogenics. As both BPs duration and other cancers were found to be risk factors for ONJ, this may explain why antiangiogenics appeared as a protective factor.

In summary, based on these conflicting results, the role of antiangiogenics in ONJ development remains controversial.

5.4.2 Study strengths

5.4.2.1 Well-executed cohort stratification

Instead of different cohorts from independent studies, the present stratification had been done within a cohort of individuals recruited under the same criteria. This homogeneous setting allowed direct and fair comparison across the stratified cohorts.

Each set of the stratification had been inspired by findings from previous Chapters. The two exposed case zoledronate and alendronate cohorts were created to crosscheck the outcome in Chapter 3, which further proved that the exposed and the non-exposed types were similar. Risk factor analysis with zoledronate super-controls, their selection method outlined in Chapter 4, also yielded interesting results and demonstrated the importance of follow-up time screening in controls recruitment.

Cohort stratification has also been well supported by detailed pre-analysis, which studied the differences across stratified cohorts. It helped explain why the alendronate and zoledronate cohorts had different risk factor results as the alendronate and zoledronate users were found largely phenotypically different. Pre-analysis also helped explain why BPs duration was not found a significant risk factor in the zoledronate super-controls cohort, unlike other zoledronate cohorts, as the length of BPs therapy was found significantly longer amongst the super-controls than controls reviewed for less than 2.1 years.

There was also much consideration on the cohort sizes of the stratified cohorts. With the clinical team's effort in participant recruitment, GENVABO had a large overall cohort. It also had the largest case cohort amongst all

multivariable studies, which enabled different combinations of stratification. For the zoledronate cohorts, they all had a size larger than 300, while both alendronate cohorts were larger than 100. However, due to the small number of non-exposed cases, alendronate super-controls, as well as individuals on BPs other than alendronate and zoledronate, these cohorts were not analysed.

In addition to the five stratified cohorts, the overall non-stratified cohort was still analysed, mainly for comparison with previous studies as cohort stratification had never been performed.

In summary, cohort stratification has been well-executed in the current GENVABO study. It was also proved important in analysing ONJ risk factors, as discussed in 5.4.1.1.

5.4.2.2 Robust risk factor analysis

For all univariable, multivariable regression models, multilevel random-effects were used to account for the clustering effect attributed to the participants being recruited in seven countries. Statistical results including the ORs, estimated coefficients, their 95% CI and standard error, had all been thoroughly scrutinised and discussed.

As ONJ is a multifactorial condition, it seemed sensible to perform multivariable analyses. Therefore, similar to previous large-scale ONJ risk factor studies, multivariable technique was also performed in the current analysis, which allowed the factors to be considered together in one single model (Vahtsevanos et al., 2009; Thumbigere-Math et al., 2012).

The 12 variables analysed in GENVABO covered most of the significant risk factors identified in the eight previous multivariable studies. Factors such as obesity and denture were not included, mainly because their results remain controversial in the literature (Campisi et al., 2014). With regard to periodontal disease, a significant risk factor reported in two multivariable studies with approximately 60 to 70 participants; it was manageable to record periodontal health in such small cohorts (Thumbigere-Math et al., 2013; Tsao et al., 2013). However, in GENVABO, it would not be feasible to accurately phenotype periodontal status, a chronic condition, for all 669 participants. Nonetheless, most sound and consistent factors were analysed in the current study.

5.4.2.3 Novel pre-risk factor and post-hoc analyses

Pre-risk factor and post-hoc analyses had never been performed in any of the multivariable studies and GENVABO was the first in designing such analyses to complement ONJ risk factor studies.

Pre-risk factor analysis in the current chapter compared (i) the zoledronate users against the alendronate users, and (ii) the zoledronate super-controls against controls reviewed for less than 2.1 years. Together with the comparison between the exposed and the non-exposed type ONJ in Chapter 3, these pre-analyses served to explain the risk factor results in the overall and all five stratified cohorts. Similar to Chapter 3, the current pre-risk factor analysis made thorough comparisons with regard to over 10 phenotypic features. There were both descriptive and inferential statistics. A bar chart was plotted to illustrate the difference in percentages for the variables. Multilevel random-effects were also used in all univariable regressions to account for the clustering effect.

As for post-hoc analysis, association between all significant risk and potential factors were checked and a total of six multilevel random-effects univariable regression tests were performed. There were also both descriptive and inferential statistics. A graph was plotted to visualise the positive and significant association between age and BPs duration. Table 49 was also constructed to summarise the interrelationship of all significant factors. Multilevel random-effects were also used in all univariable regressions to account for the clustering effect.

The key strength of post-hoc analysis has been generating additional information. For instance, both GENVABO and Sedghizadeh et al., 2013 identified age and BPs duration as risk factors. However, only GENVABO could prove that the two factors were related positively and significantly with each other. Furthermore, for factors of similar nature, it would be interesting to see if there were any interaction between each other using post-hoc analysis. The current study investigated thoroughly the relationships between other cancers, BPs duration and concomitant antiangiogenics, which were all related to underlying diseases and BPs therapy. Unfortunately, there were no such analyses in Wessel et al., 2008 and Thumbigere-Math et al., 2012; and the interrelationship between ONJ, smoking, diabetes and obesity, which were all life-style related factors, remained unknown.

5.4.3 Study limitations

5.4.3.1 Osteoporosis and alendronate patients

There were 88 less osteoporotic controls than cases. There were also 78 fewer alendronate controls than cases; and even fewer alendronate super-controls, making additional risk factor analysis with these super-controls impractical.

This may be explained by the low incidence of ONJ amongst osteoporotic and alendronate patients (Kühl et al., 2012), which may cause a low follow-up rate for ONJ screening, leading to the small number of controls available for recruitment into the GENVABO study. However, the current participants had already been recruited under a multicentre setting, which has been most effective in recruiting large number of patients.

In May 2013, a proposal was drafted and submitted to the Department of Twin Research at King's College London. It was for permission to access its database of a large registry with osteoporotic patients (Moayyeri et al., 2013). Unfortunately, Twin Research replied that they had no dental records for these patients and the proposal was rejected. Otherwise, it would have been ideal to also include these patients into the GENVABO study.

5.4.3.2 BPs duration

BPs duration was recorded and analysed as a surrogate variable for cumulative BPs dose in the current study. As there were patients prescribed with different types of BPs, this may present a problem when all participants were analysed together in the overall cohort, e.g. the same duration of zoledronate and alendronate does not imply the same cumulative BPs dose. However, it is completely different in the stratified cohorts. When the

participants were grouped according to their BPs type, their BPs duration should then be roughly proportional to their cumulative BPs dose. It was because the dose of the same BPs type is usually similar (Ruggiero et al., 2014). For instance, alendronate is usually prescribed as 10mg a day, or 70mg per week. Zoledronate is usually prescribed as 4mg per three weeks, except for few osteoporotic patients who were prescribed with 5mg per year.

With regard to phenotyping of BPs dose, it requires detailed medical records and its calculation may not be familiar to many dental clinicians. In contrast, BPs duration requires a start and an end date only, which is much more convenient and accurate in phenotyping. Moreover, the literature found that BPs duration was as strong an ONJ risk factor as BPs dose (Campisi et al., 2014). Therefore, it is considered appropriate to analyse BPs duration in replacement of cumulative BPs dose, provided that cohort stratification according to BPs type has been performed.

5.4.3.3 Systemic factors and other potential factors

Four systemic factors were studied: they were diabetes, smoking, use of steroids and antiangiogenics. Details such as the severity and control of diabetes, blood glucose level, and dosage of concomitant medications were not recorded, mainly because data collection of such information may complicate clinical phenotyping and discourage participation from clinical centres. Nevertheless, the presence or absence of diabetes history, current or previous smoking history, presence or absence of concomitant medication use had all been thoroughly phenotyped and analysed in GENVABO, which was comparable to previous ONJ risk factor studies (Wessel et al., 2008; Katz et al., 2011; Thumbigere-Math et al., 2012).

Regardless of such convenient clinical phenotyping, data for smoking were missing in 97 individuals. Fortunately, the *p*-value for smoking was greater than 0.1 at the univariable level in all six cohorts and was excluded from the final multivariable model. Therefore, the final results of these cohorts had not been affected by the missing data. On the other hand, data for diabetes, steroids and antiangiogenics were all complete.

One major limitation has been the missing data on dentoalveolar surgery history amongst the controls due to an issue with the CRF design. However, unlike smoking, dental extraction is considered a strong risk factor and is going to be present in the final multivariable model and influence the final results (Campisi et al., 2014). In response, the clinical team leaders were contacted and retrieval of such information is still in progress. Regrettably, it is impossible to include this dental factor at the time of writing. However, its analysis in GENVABO may still be possible in the near future.

As discussed in 5.4.2.2, some factors were not analysed in the current study. However, it is not possible to include every single potential factor and some weak factors may even affect the accuracy of the results. All in all, GENVABO analysed the majority of the important factors and the outcome was generally reliable and helpful in understanding the development of ONJ.

5.5 Links to other Chapters

This Chapter further supports findings from Chapter 3 that the exposed and non-exposed types are similar, i.e. the inclusion of the non-exposed cases into the all cases cohorts did not change its risk factor analysis results.

BPs duration was not found a significant risk factor in the super-control cohort, i.e. controls who had been reviewed for 2.1 years or longer, investigated in Chapter 4. This suggested that BPs duration may have been overestimated in some previous studies, possibly due to the recruitment of the inadequately reviewed controls, who were also more likely to have a shorter BPs duration.

Age, BPs duration, the use of steroids and other cancers were identified as significant risk factors in this Chapter, while the use of antiangiogenics was the only significant protective factor. Further search for genetic risk factor continues in Chapter 6.

CHAPTER 6

Genetic Risk Factors

ONJ cases versus population controls; Replication cases versus discovery cases

6.1 Introduction

6.1.1 Literature review

6.1.1.1 ADR pharmacogenetics

Inter-individual genetic variants are known to determine potential disparate responses to medications, including toxicity. It was estimated in a systematic review that genetic variability could contribute to ADR development in more than half of the medications examined (Phillips et al., 2001). Examples of genetic factors contributing to individuals' susceptibility to ADR include *HLA-B*15:02* for carbamazepine-induced Stevens-Johnson syndrome (SJS) (Chung et al., 2004), *SLCO1B1* for statin-induced myopathy (Link et al., 2008), and *HLA-B*57:01* for abacavir-induced hypersensitivity reactions (Hetherington et al., 2002; Mallal et al., 2002).

Therefore, in addition to clinical risk factors, genetic variants may also contribute to ONJ development. In the past few years, there have been a number of pharmacogenetic studies on ONJ (Sarasquete et al., 2008; English et al., 2010; Katz et al., 2011; Arduino et al., 2011; Di Martino et al., 2011; Marini et al., 2011; Such et al., 2011; Nicoletti et al., 2012; La Ferla et al., 2012; Balla et al., 2012; Stockmann et al., 2013).

Due to wider genome coverage and the advantage of being hypothesis-free, GWAS are considered more powerful than candidate gene studies (Tabor et al., 2002; Kraft et al., 2009). However, in ONJ, there are currently only two GWAS (Sarasquete et al., 2008; Nicoletti et al., 2012). Possibly due to their relatively small cohort sizes, no genome-wide significant variants were identified.

6.1.1.2 Extreme phenotyping

Having small cohorts also made extreme phenotyping impossible. It refers to focusing on individuals with extreme phenotype, i.e. more extreme disease behaviour, so as to enhance the efficiency in identifying genetic variants (Li et al., 2011).

Extreme phenotyping has been commonly practised in ADR GWAS. In the case of statin-induced myopathy, there were separate analyses according to myopathy type (Link et al., 2008). The OR of *SLCO1B1* rs4149056 amongst patients with incipient myopathy, who may or may not have muscle symptoms, was 9.6. Whereas in definite myopathy, all are presented with muscle symptoms and the OR became much higher (OR=27.2). In another GWAS on phenytoin-related severe cutaneous adverse reactions, the OR of *CYP2C9*3* was 11.96 amongst the SJS and toxic epidermal necrolysis patients (Chung et al., 2014). This was slightly higher than the OR amongst the less extreme DRESS patients, i.e. drug reactions with eosinophilia and systemic symptoms (OR=9.22).

In a study on carbamazepine-induced skin reactions, extreme phenotyping was also performed and only SJS patients were analysed (Chung et al., 2004). It yielded a very strong marker, *HLA-B*15:02* (OR=895). As a result, its screening prior to carbamazepine therapy is now recommended by the FDA (Leckband et al., 2013).

Prior to GENVABO, extreme phenotyping had never been attempted in GWAS on ONJ, which may further explain why no genome-wide significant variants were identified (Sarasquete et al., 2008; Nicoletti et al., 2012).

6.1.1.3 Replication study

Another important aspect of GWAS is the need to replicate results in an independent population with similar phenotype. This is considered the gold standard in minimising potential technical or methodological bias, leading to any spurious association signal in the discovery GWAS (McCarthy et al., 2008; Bush and Moore, 2012).

In the literature, it was found that replication studies had also been routinely performed in ADR GWAS. For instance, Link et al., confirmed the association of *SLCO1B1* with statin-induced myopathy in its candidate gene replication study. There was also replication to support its discovery GWAS results in phenytoin-related severe cutaneous adverse reactions (Chung et al., 2014).

In ONJ, four candidate gene studies attempted to replicate association with rs1934951 in gene *CYP2C8* suggested by Sarasquete et al., 2008 (English et al., 2010; Katz et al., 2011; Such et al., 2011; Balla et al., 2012). However, participants in these studies had different phenotype from the discovery GWAS, regarding ethnicity, underlying disease and BPs history. Therefore, none found *CYP2C8* a significant genetic risk factor. On the other hand, there remains no attempted replication for gene *RBMS3* suggested by Nicoletti et al., 2012.

6.1.2 Objectives

The objective is to investigate ONJ genetic risk factors, in a large, multicentre, well-phenotyped cohort, coupled with extreme phenotyping. The other objective is to test whether the replication cohort cases are phenotypically similar to the discovery cohort cases.

6.2 Methods

This part of the study involves GWAS discovery analysis, designed and performed by the GENVABO genetic team. Later, the replication case cohort was compared with the discovery case cohort, designed and performed by the author from the clinical team.

6.2.1 GWAS discovery analysis

6.2.1.1 Outcome

The aim of the GWAS was to investigate the association between ONJ development and genetic factors. The objective was to detect if there were any genome-wide significant genetic factors.

6.2.1.2 Statistical analysis

358 Caucasian cases were matched with 2,554 Caucasian population controls. Genotyping of SNPs and CNVs using 1,072,820 probes was performed. Associations between genetic variants and ONJ were tested using logistic regression and Fisher's Exact test through PLINK, a statistical software for GWAS. The genome-wide significance threshold was 5E-8. GWAS result provided by the genetic team as of 9 October 2014 is presented below in 6.3.1.

6.2.2 Replication cohort analysis

6.2.2.1 Outcomes

6.2.2.1.1 Primary outcome

The primary aim was to compare the discovery and replication case cohorts using descriptive statistics. The primary objective was to detect if there were any major numerical differences in their phenotype data.

6.2.2.1.2 Secondary outcome

The secondary aim was to compare the two cohorts using inferential statistics. The objective was to detect if there were any phenotypically, statistically significant differences between the discovery and the replication cohorts.

6.2.2.2 Statistical analysis

All analyses were performed in Stata version 12.1 (Stata Corp., College Station, TX, US).

6.2.2.2.1 Primary outcome

For numerical data, including age, BPs duration and ONJ onset time, their mean, median, standard deviation and range were calculated. For categorical data, including gender, underlying disease, BPs type and ONJ features, numbers and percentages were calculated. The percentages calculated were also plotted in a bar chart, constructed in Microsoft Excel 2013.

6.2.2.2.2 Secondary outcome

The explanatory variable was discovery case=0 and replication case=1, and each phenotypic feature formed the outcome variable.

The association between the explanatory variable and each numerical outcome variable, including age, BPs duration and onset time, was investigated with random-effects univariable linear regression. Random-effects univariable logistic regression was used for binary outcome variables, including gender, underlying disease, BPs type, dentoalveolar surgery history and ONJ type.

Multilevel random-effects were used to account for the clustering effect attributed to the participants being recruited in eight countries. The significance level for these analyses was 5%.

6.3 Results

6.3.1 GWAS results

6.3.1.1 Discovery cohort

With all 358 Caucasian cases compared with 2,554 Caucasian population controls, i.e. individuals not exposed to BPs whose anonymous genotype data had been collected in previous studies and their database was made available for research purpose, although two top SNPs were found, both were not genome-wide significant (*p*>5E-8).

Table 50 summarises the two top SNPs found and the Manhattan plot illustrates the results of the GWAS (Figure 5). The y-axis stands for the –log10 of the *p*-value and the horizontal dotted line indicates the genome-wide significance threshold, 5E-8. The x-axis records the chromosome position of the SNPs, while each dot in the plot represents a SNP. The two top SNPs at chromosomes 14 and 15, just below the horizontal dotted line, are highlighted in bright green.

Of note, there were also additional GWAS analyses with the exposed ONJ cases, non-exposed ONJ cases, zoledronate-associated and alendronate-associated ONJ cases. None of these four cohorts yielded positive results and no genome-wide significant SNP was found (*p*>5E-8).

6.3.1.2 Cohorts with extreme phenotyping

ONJ onset time and event were chosen for extreme phenotyping. Other features such as ONJ site and type were not suitable, as they do not have strong indication of disease severity. As for jawbone exposure dimension and pain intensity, these are cross-sectional data and are supposed to change over time. Therefore, early onset ONJ and non-surgery triggered ONJ, also known as spontaneous cases, were targeted.

Amongst all the cases, their ONJ onset time was sorted and the first quartile was selected (*N*=85). These early onset cases were then compared with 2,554 Caucasian population controls. A SNP, rs10277926, with high OR but borderline genome-wide significance, was identified (OR=6.28, *p*=1.69E-07) (Table 51 and Figure 6). This SNP is located at gene *BBS9* in chromosome 7.

Also relating to ONJ onset, whether or not a case had been triggered by dentoalveolar surgery prior to ONJ development was investigated. A hundred and seventy seven non-surgery triggered cases were again compared with 2,554 Caucasian population controls. For the first time, a genome-wide significant genetic risk factor was found, rs12440268, as indicated by the red dot above the dotted line in the Manhattan plot (OR=2.66, *p*=1.21E-08) (Table 52 and Figure 7). This SNP is located at gene *TJP1* in chromosome 15.

Of note, the same SNP rs12440268 was not significant in the discovery cohort GWAS when there was no extreme phenotyping.

Table 50. Discovery cohort GWAS result

SNP	Gene	Chromosome	OR	*p*-value	Frequency case	Frequency control
rs10484024	*FOXN3*	14	0.65	3.15E-07	0.3701	0.4679
rs12440268	*TJP1*	15	2.05	5.52E-07	0.1047	0.0585

Figure 5. Discovery cohort GWAS result

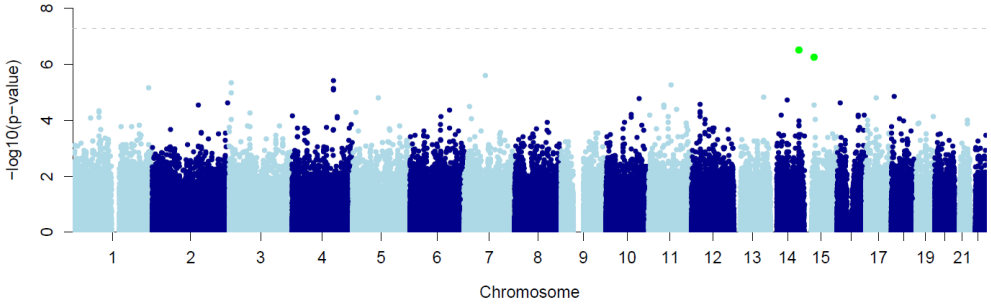


Table 51. GWAS result with ONJ onset time as extreme phenotype

SNP	Gene	Chromosome	OR	*p*-value	Frequency case	Frequency control
rs10277926	*BBS9*	7	6.28	1.69E-07	0.1111	0.0262

Figure 6. GWAS result with ONJ onset time as extreme phenotype

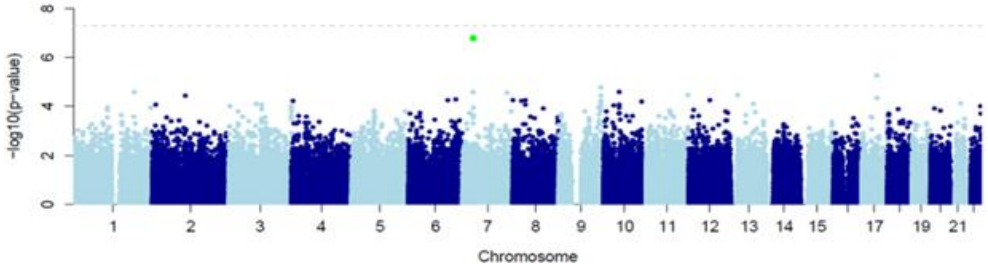
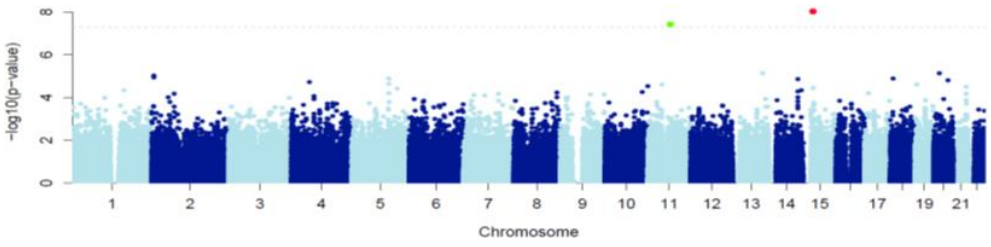


Table 52. GWAS result with ONJ onset event as extreme phenotype

SNP	Gene	Chromosome	OR	*p*-value	Frequency case	Frequency control
rs12440268	*TJP1*	15	2.66	1.21E-8	0.1356	0.0585
rs4340077	*SHANK2*	11	2.12	5.59E-8	0.2288	0.1337

Figure 7. GWAS result with ONJ onset event as extreme phenotype



6.3.2 Replication cases versus discovery cases

The replication cohort consisted of 130 new ONJ cases who had been recruited at seven centres in Hungary, Italy and the UK since October 2013.

The replication cases were mostly Caucasians (*N*=127, 97.7%) and they shared similar age, gender ratio and BPs history with the 393 discovery cases (Table 53). However, their top three underlying diseases were different. There were a smaller proportion of multiple myeloma patients but a larger proportion of metastatic cancer patients in the replication cohort. With regard to ONJ onset, the two groups shared very similar onset time, but there were nearly 20% more non-surgery-triggered ONJ in the replication cohort. The proportion of non-exposed type ONJ was also higher by approximately 10% in the replication cohort than in the discovery cohort.

The percentages calculated were also plotted in Graph 14. The major differences were with dentoalveolar surgery history (17.6%), followed by multiple myeloma (14.7%), then with the non-exposed type of ONJ (11.6%), and metastatic breast cancer (10.5%). The rest differed by less than 10%.

As for the results using univariable regressions, 13 comparisons were made and only two were found statistically significant ($p<0.05$) (Table 54). There was a statistically significantly smaller proportion of multiple myeloma patients in the replication cohort than in the discovery cohort (OR=0.38, 95% CI 0.20 to 0.71, $p=0.002$). There was also a significantly smaller proportion of exposed type ONJ amongst the replication cases (OR=0.27, 95% CI 0.15 to 0.49, $p<0.001$). In contrast, the two groups had very similar age and BPs duration as their estimated coefficients were both nearly zero.

Table 53. Replication cases versus discovery cases; descriptive statistics

		Replication cases *N* = 130		Discovery cases *N* = 393	
		n	**%**	***n***	**%**
Age, *decade*	Mean, median	6.9, 7.0		6.9, 7.0	
	SD	1.0		0.9	
	Range	4.3 to 8.8		3.7 to 8.9	
Gender	Female	99	76.2%	278	70.7%
	Male	31	23.8%	115	29.3%
Primary underlying disease	Osteoporosis	42	32.3%	137	34.9%
	Multiple myeloma	15	11.5%	103	26.2%
	Metastatic breast cancer	43	33.1%	89	22.6%
	Metastatic prostate cancer	18	13.8%	37	9.4%
	Other cancers	12	9.2%	27	6.9%
BPs with longest duration	Zoledronate	66	50.8%	230	58.5%
	Alendronate	27	20.8%	109	27.7%
BPs duration, *year*	Mean, median	3.6, 2.8		3.7, 2.8	
	SD	3.0		3.1	
	Range	0.2 to 15.1		0.1 to 19.9	
History of dentoalveolar surgery		42	32.3%	196	49.9%
ONJ onset time, *year*	Mean, median	4.2, 3.2		4.0, 3.1	
	SD	3.4		3.2	
	Range	0.2 to 15.9		0.1 to 19.9	
ONJ type	Exposed	101	77.7%	344	87.5%
	Non-exposed	28	21.5%	39	9.9%

Graph 14. Replication cases versus discovery cases; differences in percentages

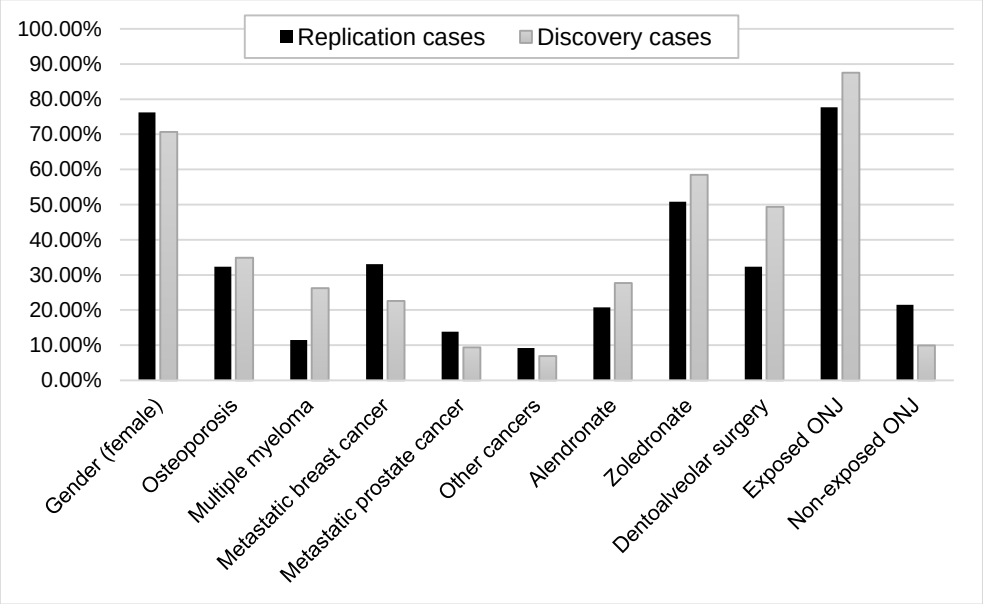


Table 54. Replication cases versus discovery cases; random-effects univariable regression

Numerical outcome variable		*N* = 523; replication case=1, discovery case=0		
		Estimated coefficient	**95% CI**	***p*-value**
Demographics	Age, *decade*	0.06	-0.17 to 0.30	0.593
BPs duration, *year*		-0.01	-0.66 to 0.63	0.971
ONJ onset time, *year*		0.21	-0.48 to 0.89	0.554
Binary outcome variable		**OR**	**95% CI**	***p*-value**
Demographics	Gender (M=0, F=1)	1.32	0.84 to 2.09	0.234
Primary underlying disease	Osteoporosis	1.28	0.75 to 2.18	0.361
	Multiple myeloma	0.38	0.20 to 0.71	0.002*
	Metastatic breast cancer	1.23	0.70 to 2.18	0.475
	Metastatic prostate cancer	1.55	0.85 to 2.82	0.156
	Other cancers	1.38	0.67 to 2.86	0.383
BPs with longest duration	Alendronate	0.88	0.49 to 1.57	0.662
	Zoledronate	0.78	0.48 to 1.27	0.320
History of dentoalveolar surgery		0.96	0.58 to 1.58	0.870
ONJ type (non-exposed=0, exposed=1)		0.27	0.15 to 0.49	<0.001*

* Significant results, $p<0.05$

```
Random-effects ML regression                    Number of obs      =       520
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      65.0
                                                               max =       315

                                                LR chi2(1)         =      0.29
Log likelihood  = -709.79303                    Prob > chi2        =    0.5915

------------------------------------------------------------------------------
   agedecade |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |   .0635444    .118905     0.53   0.593    -.1695052     .296594
       _cons |   6.918775   .1038517    66.62   0.000     6.715229    7.122321
-------------+----------------------------------------------------------------
    /sigma_u |   .2151734   .1121535                      .0774679    .5976611
    /sigma_e |     .93945   .0294764                      .8834178    .9990361
         rho |   .0498453   .0497372                      .0047449    .2423313
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    3.68 Prob>=chibar2 = 0.028




Random-effects ML regression                    Number of obs      =       500
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      62.5
                                                               max =       315

                                                LR chi2(1)         =      0.00
Log likelihood  = -1270.7593                    Prob > chi2        =    0.9709

------------------------------------------------------------------------------
 bpsduration |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |  -.0120185   .3290896    -0.04   0.971    -.6570223    .6329853
       _cons |   3.700783   .2145834    17.25   0.000     3.280207    4.121359
-------------+----------------------------------------------------------------
    /sigma_u |   .2578408          .                             .           .
    /sigma_e |   3.067509   .0972623                     2.882681    3.264188
         rho |   .0070157          .                             .           .
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    1.08 Prob>=chibar2 = 0.149
```

```
Random-effects ML regression                    Number of obs      =       486
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      60.8
                                                               max =       313

                                                LR chi2(1)         =      0.35
Log likelihood  = -1258.7426                    Prob > chi2        =    0.5545

------------------------------------------------------------------------------
onsettimeyear |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
    disc0rep1 |   .2071783   .3503873     0.59   0.554    -.4795681    .8939247
        _cons |   4.045819   .2408647    16.80   0.000     3.573733    4.517905
--------------+---------------------------------------------------------------
     /sigma_u |   .3032485          .                           .           .
     /sigma_e |   3.217667   .1035807                     3.020925    3.427223
          rho |   .0088039          .                           .           .
------------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=    0.78 Prob>=chibar2 = 0.188
```

```
Random-effects logistic regression              Number of obs      =       523
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      65.4
                                                               max =       317

                                                Wald chi2(1)       =      1.42
Log likelihood  = -308.97034                    Prob > chi2        =    0.2336

------------------------------------------------------------------------------
  genderm0f1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |    1.32108    .308857     1.19   0.234     .8354596    2.088972
       _cons |   2.417955   .2681004     7.96   0.000     1.945664     3.00489
-------------+----------------------------------------------------------------
    /lnsig2u |  -14.64457    63.1747                     -138.4647    109.1756
-------------+----------------------------------------------------------------
     sigma_u |   .0006607   .0208682                     8.57e-31    5.10e+23
         rho |   1.33e-07   8.38e-06                     2.23e-61           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       523
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      65.4
                                                               max =       317

                                                Wald chi2(1)       =      0.83
Log likelihood  = -322.71431                    Prob > chi2        =    0.3614

------------------------------------------------------------------------------
          op |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |   1.280831   .3473685     0.91   0.361     .7527331    2.179427
       _cons |   .8920066   .3370086    -0.30   0.762      .425379     1.87051
-------------+----------------------------------------------------------------
    /lnsig2u |  -.1213772    .676062                     -1.446434     1.20368
-------------+----------------------------------------------------------------
     sigma_u |   .9411163   .3181265                     .4851888    1.825475
         rho |   .2121148   .1129849                     .0667772    .5032081
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    26.36 Prob >= chibar2 = 0.000
```

```
Random-effects logistic regression              Number of obs      =       523
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      65.4
                                                               max =       317

                                                Wald chi2(1)       =      9.14
Log likelihood  = -272.52902                    Prob > chi2        =    0.0025

------------------------------------------------------------------------------
          mm |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |   .3819533   .1215663    -3.02   0.002     .2046887    .7127325
       _cons |   .2931688   .1063026    -3.38   0.001     .1440367    .5967086
-------------+----------------------------------------------------------------
    /lnsig2u |  -2.434049   2.691291                     -7.708882    2.840784
-------------+----------------------------------------------------------------
     sigma_u |   .2961099    .398459                      .0211854    4.138743
         rho |     .02596   .0680521                      .0001364    .8388825
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.05 Prob >= chibar2 = 0.408
```

```
Random-effects logistic regression              Number of obs      =       523
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      65.4
                                                               max =       317

                                                Wald chi2(1)       =      0.51
Log likelihood  = -290.40188                    Prob > chi2        =    0.4752

------------------------------------------------------------------------------
         mbc |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |    1.23112   .3584796     0.71   0.475     .6957385    2.178485
       _cons |   .2112751   .0743994    -4.41   0.000     .1059503     .421303
-------------+----------------------------------------------------------------
    /lnsig2u |  -.6950247   .9463805                     -2.549896    1.159847
-------------+----------------------------------------------------------------
     sigma_u |   .7064433   .3342821                      .2794454    1.785902
         rho |   .1317158   .1082345                      .0231861    .4922505
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     4.71 Prob >= chibar2 = 0.015
```

```
Random-effects logistic regression              Number of obs      =       523
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      65.4
                                                               max =       317

                                                Wald chi2(1)       =      2.01
Log likelihood  = -174.90879                    Prob > chi2        =    0.1559

------------------------------------------------------------------------------
         mpc |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |   1.546246   .4748876     1.42   0.156      .846945    2.822943
       _cons |   .1039301   .0179521   -13.11   0.000     .0740816    .1458048
-------------+----------------------------------------------------------------
    /lnsig2u |  -15.59693   65.59378                     -144.1584    112.9645
-------------+----------------------------------------------------------------
     sigma_u |   .0004104   .0134587                      4.97e-32    3.39e+24
         rho |   5.12e-08   3.36e-06                      7.51e-64           1
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.00 Prob >= chibar2 = 1.000
```

```
Random-effects logistic regression              Number of obs      =       523
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      65.4
                                                               max =       317

                                                Wald chi2(1)       =      0.76
Log likelihood  = -138.30473                    Prob > chi2        =    0.3835

------------------------------------------------------------------------------
          oc |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |   1.382564   .5138943     0.87   0.383      .667262    2.864665
       _cons |   .0664944   .0228191    -7.90   0.000     .0339372     .130285
-------------+----------------------------------------------------------------
    /lnsig2u |  -3.118112   3.288697                     -9.56384    3.327616
-------------+----------------------------------------------------------------
     sigma_u |   .2103345   .3458633                      .0083799    5.279377
         rho |   .0132691    .043059                      .0000213    .8944259
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     0.14 Prob >= chibar2 = 0.353
```

```
Random-effects logistic regression              Number of obs      =       523
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      65.4
                                                               max =       317

                                                Wald chi2(1)       =      0.19
Log likelihood  = -293.31582                    Prob > chi2        =    0.6620

------------------------------------------------------------------------------
         ale |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |   .8784757   .2603917    -0.44   0.662     .4913856    1.570497
       _cons |   .4951851   .1428485    -2.44   0.015     .2813308    .8716014
-------------+----------------------------------------------------------------
    /lnsig2u |  -.8184197   .7972496                      -2.381    .7441608
-------------+----------------------------------------------------------------
     sigma_u |   .6641748   .2647566                      .3040692     1.45075
         rho |   .1182334   .0831166                      .0273356    .3901488
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    10.28 Prob >= chibar2 = 0.001
```

```
Random-effects logistic regression              Number of obs      =       523
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      65.4
                                                               max =       317

                                                Wald chi2(1)       =      0.99
Log likelihood  = -346.56035                    Prob > chi2        =    0.3198

------------------------------------------------------------------------------
         zol |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |   .7796776   .1950586    -0.99   0.320     .4774882    1.273114
       _cons |   .8177601    .243927    -0.67   0.500     .4557469    1.467331
-------------+----------------------------------------------------------------
    /lnsig2u |  -.7700118   .7404435                     -2.221254    .6812307
-------------+----------------------------------------------------------------
     sigma_u |   .6804466   .2519161                      .3293523    1.405812
         rho |   .1233741   .0800811                      .0319194    .3752834
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    20.40 Prob >= chibar2 = 0.000
```

```
Random-effects logistic regression              Number of obs      =       513
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      64.1
                                                               max =       313

                                                Wald chi2(1)       =      0.03
Log likelihood  = -327.00548                    Prob > chi2        =    0.8695

------------------------------------------------------------------------------
dentoalveolarsurgery |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
---------------------+--------------------------------------------------------
           disc0rep1 |   .9588694   .2452068    -0.16   0.870     .5808795    1.582825
               _cons |   .4789111    .279119    -1.26   0.207      .152812    1.500902
---------------------+--------------------------------------------------------
            /lnsig2u |   .8027772   .7004123                     -.5700058     2.17556
---------------------+--------------------------------------------------------
             sigma_u |   1.493898   .5231722                      .7520121    2.967679
                 rho |   .4041819   .1686725                      .1466835    .7280423
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    40.50 Prob >= chibar2 = 0.000




Random-effects logistic regression              Number of obs      =       512
Group variable: country                         Number of groups   =         8

Random effects u_i ~ Gaussian                   Obs per group: min =         7
                                                               avg =      64.0
                                                               max =       312

                                                Wald chi2(1)       =     18.80
Log likelihood  = -179.77165                    Prob > chi2        =    0.0000

------------------------------------------------------------------------------
    typen0e1 |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   disc0rep1 |   .2733811     .08178    -4.34   0.000      .152103    .4913593
       _cons |   38.07222   30.32796     4.57   0.000     7.990047    181.4124
-------------+----------------------------------------------------------------
    /lnsig2u |   .7917122   .9892541                     -1.14719    2.730614
-------------+----------------------------------------------------------------
     sigma_u |   1.485655   .7348454                      .563496    3.916926
         rho |   .4015201   .2377194                     .0880213    .8234309
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =    27.51 Prob >= chibar2 = 0.000
```

6.4 Discussion

6.4.1 Main findings

6.4.1.1 TJP1 gene

SNP rs12440268 was found to be genome-wide significant (p<5E-8). This SNP is related to gene *TJP1*, which encodes protein at the tight junctions. Tight junctions are intercellular junctions in simple epithelia and endothelia, as well as stratified squamous epithelia including the gingivae, lingual and other types of oral mucosa (Franke and Pape, 2012). Their main purpose is to maintain epithelial stratification and integrity, which defends the body against any pathogens, toxins and allergens (Brandner et al., 2003).

To date, there are no previous studies investigating the status of tight junctions amongst those diagnosed with ONJ. A recent study, which investigated the effect of alendronate on human oral mucosa, reported that epithelial integrity was unaffected amongst ONJ-free individuals who were managed with alendronate (Donetti et al., 2014). This only suggests that BPs *per se* may have no effect on tight junctions.

However, it may be different under the influence of genetic variants, such as rs12440268. In fact, the association of ONJ with *TJP1* further supported the pathogenesis hypothesis of impaired wound healing. In other words, individuals with this polymorphism may have “less protective” oral mucosa and hence poorer healing ability than those not presented with the polymorphism.

Nonetheless, this marker had a relatively small OR (OR=2.66), and it was significant amongst the 177 non-surgery triggered cases only, but not the entire cohort of 358 cases.

6.4.1.2 BBS9 gene

rs10277926, though not genome-wide significant, had the largest OR amongst all identified top SNPs (OR=6.28, *p*=1.69E-07). This SNP is related to gene *BBS9*, also known as *PTHB1*, which stands for parathyroid hormone responsive-B1 (Nishimura et al., 2005). It is reported that this gene is down-regulated by parathyroid hormone in osteoblasts (Kang et al., 2011a).

BBS refers to Bardet-Biedl syndrome, a genetic disorder caused by *BBS* gene mutation. The syndrome does have a link with the jawbone; features including jawbone atresia, high arched palate and malocclusion have been reported (Majumdar et al., 2012; Ferreira do Amaral et al., 2014). Moreover, association of *BBS9* gene with nonsyndromic sagittal craniosynostosis, a congenital anomaly with skull development, has also been reported in a previous GWAS (Justice et al., 2012).

In other words, individuals with this polymorphism may have different bone remodelling activity, and even different jawbone phenotype, from those not presented with the polymorphism. Nonetheless, at this stage, this marker is not found statistically significant and further confirmation is required.

6.4.1.3 Replication cohort

Power calculation by the genetic team suggested 200 cases to be recruited for replication. Currently, 65% of the target has been met. The discovery and replication case cohorts are supposed to be phenotypically similar. At present, the two groups share very similar ethnicity, age and BPs duration, and similar gender proportion, BPs type, ONJ onset time and event. The major differences are with underlying disease and ONJ type, i.e. the replication cohort has a smaller proportion of multiple myeloma cases as well as exposed type ONJ.

6.4.2 Study strengths

6.4.2.1 Collaborative teamwork

There was a dedicated clinical team, consisting of specialists in Oral Medicine, Oral and Maxillofacial Surgery, Oncology, Haematology, Rheumatology and Radiology, who played a key role in recruiting and adjudicating GENVABO participants. The team also included the non-exposed cases and proved that they were comparable to the exposed ones. In addition to recruiting a large cohort of ONJ patients, another contribution was the detailed analysis of clinical phenotype. There were also robust analyses of the clinical risk factors to complement the genetic risk factor results.

The genetic team has great experience in pharmacogenetics and has previously successfully identified genetic variants associated with drug-related liver toxicity (DILI) and serious skin rashes (SSR) (Holden et al., 2014). Their main contributions were genotyping, matching the ONJ cases with population controls, and very importantly, designing and performing robust GWAS analyses.

The two teams worked closely together and had regular communications including emails, teleconferences and visits to each other's office. This facilitated the planning of the first extreme phenotyping in ONJ GWAS, resulting in the very first genome-wide significant SNP for ONJ development.

6.4.2.2 Large and well-phenotyped discovery cohort

Compared with previous GWAS on ONJ (Sarasquete et al., 2008; Nicoletti et al., 2012), GENVABO is the largest. There were 23 centres from eight countries, and 393 ONJ cases were recruited, including non-exposed ONJ.

Three hundred and fifty eight Caucasian cases were analysed, which was nearly 12 times more than in previous studies and thus providing more adequate power (Jorgensen and Williamson, 2008).

Clinical phenotyping was also more thorough than the other two ONJ GWAS. Previous studies provided minimal phenotype data, while the current study collected more detailed information regarding medical and dental history, in particular, ONJ onset time and event, which enabled extreme phenotyping and the discovery of a genome-wide significant SNP for ONJ development.

6.4.2.3 Large and well-phenotyped replication cohort

This is going to be the first replication cohort recruited by the same consortium under the same setting as the discovery cohort. This will facilitate comparison of results between the two cohorts in future.

In replicating GWAS result from Sarasquete et al., 2008, four studied gene *CYP2C8* and the largest study recruited only 46 ONJ cases (English et al., 2010; Katz et al., 2011; Such et al., 2011; Balla et al., 2012). With a size of 130 cases, and 70 more to be recruited, GENVABO's replication cohort is going to be the largest amongst all reported ONJ pharmacogenetic studies.

The same standardised CRF was used for the replication cases and they had also been thoroughly phenotyped. The two groups had also been rigorously compared using both descriptive and inferential statistics and were found largely phenotypically similar. In addition, it is favourable to have a larger proportion of non-surgery triggered cases in the replication cohort for extreme phenotyping in the ongoing replication.

6.4.3 Study limitations

6.4.3.1 Population controls

In GENVABO, population controls were analysed instead of BPs-exposed controls. If these healthy individuals were to be exposed to BPs, depending on the potency, some would have ONJ developed. This indicates that analysis with population controls may not be completely reliable.

However, ONJ has a relatively low incidence; therefore, if exposed to BPs, there would only be a small number of potential ONJ cases amongst the population controls (Kühl et al., 2012). Second, analysis with population controls has been commonly practised in ADR GWAS. Three thousand six hundred and fifty five population controls were involved in a recent study on phenytoin-related severe cutaneous adverse reactions (Chung et al., 2014). There were also two GWAS on DILI with 3,001 and 532 population controls analysed respectively (Lucena et al., 2011; Urban et al., 2012).

In short, analysis with population controls is considered appropriate for a discovery ADR GWAS. Nonetheless, at a later stage, BPs-exposed controls will also be analysed in GENVABO. At present, DNA extraction had been completed for all controls; genotyping and statistical analyses will soon follow.

6.4.3.2 Genome coverage

There are over 10 million SNPs in human (Wangkumhang et al., 2007). In GENVABO, only a million were covered. This is because these genotyped SNPs have the property of being proxies to the untyped SNPs within the same genomic region, known as linkage disequilibrium (LD) (Roses, 2000). In other words, through LD, these one million SNPs can help predict the rest of the

genome. Therefore, to date, GWAS remains commonly performed in search of genetic variants for ADR (Daly, 2010, 2012). Nevertheless, in future, follow-up “fine-mapping” around the genome-wide significant SNP after its replication, so as to identify any previously untyped potential genetic risk factors may follow.

CHAPTER 7

Conclusion; Future Work

7.1 Conclusion

The GENVABO study involves the largest and the most comprehensive GWAS to date, accompanied by robust case cohort validation in supporting the inclusion of the non-exposed ONJ cases into the GWAS analysis, novel BPs-exposed controls cohort validation and super-controls selection, followed by sophisticated clinical risk factor analyses. In total, 523 BPs-associated ONJ cases, 276 BPs-exposed controls and 2,554 ethnicity-matched population controls were analysed.

For the first time, a genome-wide significant genetic factor was identified, and the GENVABO study also confirmed that BPs-associated ONJ is a multifactorial disease. The factors are rs12440268, a variant at gene *TJP1*, and clinical risk factors including advanced age, longer BPs duration, other cancers, mainly kidney and lung cancers as underlying diseases for BPs, and the use of steroids as concomitant medication.

These findings are not only applicable to the mainstream exposed typed ONJ, but also the non-exposed variant, which was, for the first time, included into an ONJ GWAS after thorough case validation. One may argue that the inclusion of the non-exposed cases may increase the heterogeneity of the study cohort. However, in Chapter 3, they were found largely phenotypically similar to the exposed type in terms of demographics, medical and dental history. Furthermore, in Chapter 5, with novel risk factor analyses in different stratified cohorts, it was found that the all cases cohorts, i.e. with both exposed and non-exposed cases, shared exactly the same risk factors with the cohorts consisting of exposed cases only. In other words, the inclusion of the non-

exposed cases into the all cases cohorts did not change the risk factor results. Having a larger cohort certainly increases the power of the current study and the current findings also confirm the importance of the recent revision of the 2014 AAOMS ONJ definition in which the non-exposed variant was finally included.

Time to onset amongst the ONJ cases and follow-up time of the controls were also studied. In general, in the current study, follow-up time was found shorter than time to onset, while onset time for zoledronate cases was found shorter than those on alendronate, which can be explained by the higher potency of zoledronate.

Through detailed comparison between time to onset and follow-up time, an original and novel control cohort validation was performed in Chapter 4. After careful consideration, the median onset times, 2.1 years for zoledronate and 6.0 years for alendronate, were chosen as the cut-off in selecting super-controls, i.e. those who had been more adequately reviewed and thus carry a lower risk of being “false-controls”. Out of a total of 203 zoledronate-exposed controls, 88 super-controls, who had been reviewed for 2.1 years or longer, were selected and had additional risk factor analysis in Chapter 5. Since the number of alendronate super-controls were small (*N*=8), additional analysis was not arranged due to statistical consideration.

These super-controls were also found to have longer BPs duration than the rest of the controls. In their risk factor analysis in Chapter 5, BPs duration was not found a significant ONJ risk factor in this particular cohort with super-controls, in contrast to most previous studies with no control cohort validation.

Following the investigation of the ONJ risk factors, for the first time, the interrelationship between the significant factors was also examined in the current post-hoc analysis in Chapter 5. Interestingly, there was a positive correlation between BPs duration and age, both identified as significant risk factors in the current study. In the super-control cohort, advanced age remained as a significant risk factor, however, BPs duration was no longer significant, as discussed above. This suggested that age was unlikely to be a confounder and may have direct association with ONJ. However, age was often matched between cases and controls, or adjusted in the statistical models in previous studies. The mechanism of how age relates to ONJ development is also largely unknown as this has never been formally studied.

Meanwhile, the use of steroids was also found a risk factor amongst the alendronate users. However, this remains preliminary as it showed a wide 95% confidence interval, as well as a large standard error, due to the small number of steroid users. Similarly, other cancers as a risk factor also remains preliminary as there were also only a small number of individuals diagnosed with other cancers, hence its wide 95% confidence interval and large standard error. On the other hand, the following factors were not found significant in the current GENVABO study: gender, multiple myeloma and metastatic cancers, use of zoledronate, diabetes and smoking habit.

Clinical features have also been helpful in extreme phenotyping in the current GWAS, which refers to focusing on individuals with more extreme disease behaviour and may help enhance the efficiency in identifying genetic variants. Amongst the 85 early onset cases, a genetic risk factor with large odds ratio was identified, though not genome-wide significant (OR=6.28, p=1.69E-07).

As for the said significant variant, rs12440268, it was found in the GWAS amongst 177 non-surgery triggered cases (OR=2.66, *p*=1.21E-08). Of note, the same SNP was not found significant in the overall cohort without extreme phenotyping. The two polymorphisms were related to gene *TJP1* and *BBS9*, which may support the pathogenesis hypotheses of impaired wound healing and bone remodelling inhibition, as they are associated with oral mucosa epithelial stratification and integrity, and parathyroid hormone activity.

Replication study is in progress to confirm the discovery GWAS results. The replication cases are largely phenotypically comparable to the discovery cases with regard to demographics and BPs history. There is also a large proportion of non-surgery triggered cases in the replication cohort, which is favourable to extreme phenotyping in the ongoing replication study.

In conclusion, GENVABO has brought a new perspective to the challenging research into ONJ. The non-exposed variant, never included in a GWAS before GENVABO, was found largely similar to the exposed counterpart. The effect of BPs therapy duration, traditionally believed to increase the risk of ONJ, may have been overestimated due to the absence of control validation in previous studies. Yet, advanced age, a rarely investigated phenotype, was found a significant risk factor in the current study. Lastly, possibly due to small cohort sizes, no significant genetic polymorphisms had been identified in previous studies. GENVABO performed a large GWAS with extreme phenotyping, which has led to the discovery of the first genome-wide significant SNP in supporting the role of genetic predisposition in ONJ pathophysiology. Recruitment of replication cases is ongoing.

7.2 Future work

The current study includes one of the early works on the comparison of the two ONJ types. As the non-exposed type was only recently incorporated into the official ONJ definition, it is expected that more non-exposed cases will be reported (Ruggiero et al., 2014). This is considered favourable as the previous cohort sizes of the non-exposed type had been relatively small (Schiodt et al., 2014; Fedele et al., 2015). Not only is this going to benefit ONJ type comparison studies, but also clinical and genetic risk factors analyses as there will be a larger cohort leading to more reliable results.

There was also control validation through pioneering follow-up time assessment in GENVABO. At present, the super-controls, who had been reviewed longer, showed different clinical risk factor results from controls with no follow-up time screening. In future, there should also be genetic risk factor analyses with super-controls as they are less likely to be “false-controls” and is going to yield even more robust genetic variants. In the current study, the median time to ONJ onset was chosen as the cut-off in selecting super-controls. Since this has been a first attempt, more studies in experimenting different cut-off thresholds are recommended.

Following the discussion in Chapter 5, the recruitment of a larger cohort of osteoporosis and alendronate patients, the retrieval of missing data, and the inclusion of any unanalysed clinical risk factors would be desirable in future.

It has also been suggested that ONJ development involves gene-environment interaction; therefore, clinical and genetic risk factors should be analysed hand in hand in future (Izzotti et al., 2013). Specifically, both clinical and genetic risk

factors will be entered together into a single regression model as explanatory variables, while ONJ case or control remains as outcome variable. It will then estimate the contribution of all the factors towards disease development, e.g. together, age, BPs duration and two genetic variants account for x% of ONJ occurrence (Sconce et al., 2005; Moreau et al., 2014). Statistical interaction between the clinical and genetic factors can also be investigated, and all these findings are going to be helpful in understanding ONJ pathogenesis.

In addition to the replication study and GWAS with BPs-exposed controls discussed in Chapter 6, other potential future studies may include GWAS on ONJ in association with medications other than BPs and in ethnicity groups other than Caucasians.

This is because in the majority of cases, ADR genetic variants are drug-specific. For instance, both flucloxacillin and amoxicillin-clavulanate induce liver injury, but their genetic risk factors are different from each other (Daly et al., 2009; Lucena et al., 2011). Hence there may be different sets of genetic factors for bevacizumab- and sunitinib-associated ONJ, as well as with denosumab, another antiresorptive which can also causes ONJ (Epstein et al., 2013; Sivolella et al., 2013).

Similarly, ADR genetic variants are also population- or ethnicity-specific. For example, the Asians and Europeans are presented with different genetic polymorphisms for carbamazepine-induced skin reactions, the same phenotype (Chung et al., 2004; McCormack et al., 2011). Different results are therefore expected from ONJ pharmacogenetic studies on populations other than Caucasians.

In the long term, functional study, for instance with knockout mice technique, may also be considered to test how the culprit genes behave in relation to ONJ pathogenesis (Clarke et al., 2014). Lastly, a cost-effectiveness trial can test whether the saving on the cost of ONJ management, in particular jaw surgery and medications, and patients' quality of life, can outweigh the cost of genetic screening prior to BPs prescription (Hughes et al., 2004). Once the replication is complete, the planning for these studies can follow, depending on the overall GWAS results and the availability of research funding.

CHAPTER 8

Appendix

8.1 Participant information sheet

University College London Hospitals NHS
NHS Trust

Version 3 18 April 2010

Eastman Dental Hospital
256 Grays Inn Road
London WC1X 8LD

Telephone: 020 7915 1000

Participant Information Sheet

STUDY ON THE RISK FACTORS OF JAWBONE DISEASE (OSTEONECROSIS) ASSOCIATED WITH THE USE OF BISPHOSPHONATE DRUGS

[SCIENTIFIC TITLE: BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE JAWS A CASE-CONTROL GENETIC STUDY]

Please read this sheet carefully. Please ask if you do not understand or would like more information

1. Invitation to participate
You are being asked to participate in a research project being conducted with the approval of the UCLH Ethics Committee Alpha. You have been selected as a potential participant because you might have the appropriate oral condition that we are studying. If you do not have the condition we are studying, you have been invited to participate as our study aims at comparing individuals having this condition with those who do not have it. The following information is provided so that you can make an informed decision regarding your willingness to participate. Please discuss with family and friends and ask us if there is anything which is not clear or if you would like more information.

2.Background and purpose
Bisphosphonates (BP) are drugs that in certain situations can help to protect your bones against some of the effects of cancer and osteoporosis, such as pain and weakness. In the last few years a new adverse side effect of BP drugs has been reported. This complication consists of a disease of the jawbones called "osteonecrosis of the jaws". This is a condition in which bone in the lower or upper jaw becomes exposed through the gums. Infection usually follows and the area can become swollen and painful. Jawbone disease associated with bisphosphonates can become a long-lasting problem in many of those who develop it and can be very difficult to control. Current information suggests that this condition appears to occur infrequently in patients with cancer (5-10%) and rarely in patients with benign conditions (such as osteoporosis) (0.1-0.5%) who are being treated with bisphosphonate medications. We are currently investigating the reasons why some of the individuals who use bisphosphonates develop this bone disease whilst others (the majority) do not. The aim of this study is to find out whether some individuals could be predisposed to this complication because of differences in their genes. Our target is therefore to detect possible genetic changes that could increase the risk of developing jawbone disease (osteonecrosis) associated with bisphosphonates drugs. This study will help understanding the possible risk factors of this complication and will help identifying potential preventive and therapeutic measures.

3. Alternatives
It is up to you to decide whether you want to take part in this study or not. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. We will also contact your GP and your dentist to inform them regarding your participation in the study if you agree so.

4. Study procedures
A total of 800 individuals will participate in this study. 200 will be individuals using bisphosphonates drugs and with associated jawbone disease (osteonecrosis) and 600 will be

Participant's Initials_____

Eastman Dental Hospital
256 Grays Inn Road
London WC1X 8LD

Telephone: 020 7915 1000

individuals using bisphosphonates drugs but without any sign of jawbone disease. The study consists of only one visit. Your weight and height will be recorded, your medical history will be taken, and a clinical examination of the mouth will be performed in order to detect signs of jawbone disease. 30 ml (5 small tubes) of blood will also be taken from your arm for genetic testing.

5. Risks and discomforts
Blood collection is a routine procedure which may cause you some discomfort. Clinical examination of the mouth is usually not associated with any discomfort.

6. Possible Benefits to Participants

During the study it is possible that clinicians may find signs of this jawbone disease also in individuals who were unaware of it (because of absence of pain, jaw swelling etc). This would lead to an early diagnosis and therapy (where required).

7. Confidentiality
The Investigator (study doctor) will make every possible effort to keep your personal information confidential. All the information collected will be kept by the research coordinator. The Chief Investigator is responsible for safety and security of the data. Medical records which identify you and the consent form signed by you may be inspected by an Institutional Review Board or Ethical Review Committee. The results of this research project may be presented at meetings or in publications; however, any research data released or published will not identify volunteers by name. All data and results will be completely anonymised and it will be impossible to identify you from them.

8. Genetic analysis
An analysis of genetic factors will be performed from the blood we collect from your arm. Each sample is a gift and will be coded by number. It is specified that no name will be used and hence the blood samples will be fully anonymised. Some of the samples collected may be stored after the end of the project and more laboratory tests may be performed following the first results of the study. Also in this case only numbers will be used and the blood samples will be fully anomymised

9. Complaints
If you have any complaints about study-related issues, you have the right to complain through the UCLH complaints procedure. If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, you may have grounds for legal action but you may have to pay for it.

10. Study results
The results of this research study may be presented at meetings and may be published, likely at least one year after the end of the project. No patients will be identified in any report. If you would like to receive the results of the study, please contact the Chief Investigator, Prof. S. Porter, by letter.

11. Voluntary Participation and Right to Refuse or Withdraw
Your participation in this study is voluntary. You can take as much time as you need to decide upon taking part in this study. You may refuse to participate or may discontinue participation from this study at any time without affecting your treatment. All information about your medical records will be treated as strictly confidential and will only be used for medical purposes. Your medical records may be inspected by competent authorities and properly authorised persons,

Participant's Initials_____

Eastman Dental Hospital
256 Grays Inn Road
London WC1X 8LD

Telephone: 020 7915 1000

but if any information is released this will be done in a coded form so that confidentiality is strictly maintained. Participation in this study will in no way affect your legal rights.

12. Ethic approval of the study
This study has been reviewed and approved by the joint UCL/UCLH Committee on the Ethics of Human Research (Committee Alpha).

13.Whom to Ask Questions Regarding this Study
You have the right to ask questions concerning this study at any time, and you are urged to do so. You will be informed of any significant new information pertaining to your safety. If you have any questions concerning this study, or would like to report any research related injuries please contact **Prof. Stephen Porter** at 020 7915 1000 or **Dr Stefano Fedele at** 020 7915 1004 or **Dr. Francesco D'Aiuto** at 020 7915 2334.

A copy of this information sheet and a signed consent form will be given to you.

Participant's Initials_____

8.2 Consent form

University College London Hospitals NHS
NHS Trust

Eastman Dental Hospital
256 Grays Inn Road
London WC1X 8LD

Telephone: 020 7915 1000

Version 3; 18th April 2010
Study Number:
Patient Identification Number for this trial:

CONSENT FORM

Title of project:
STUDY ON THE RISK FACTORS OF JAWBONE DISEASE (OSTEONECROSIS) ASSOCIATED WITH THE USE OF BISPHOSPHONATE DRUGS

[SCIENTIFIC TITLE: BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE JAWS: A CASE-CONTROL GENETIC STUDY]

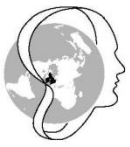
Name of Chief Investigator: **Prof. Stephen Porter**

	Please initial box
1. I confirm that I have read and understood the information sheet dated 18th April 2010 (version 3) for the above study and have had the opportunity to ask questions.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
3. I understand that sections of any of my medical notes may be looked at by the researchers and responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.	☐
4. I give permission to the investigators to pass clinical data collected from my examination to my General Practitioner and General Dental Practitioner, and to inform them of my participation in the study.	☐
5. I understand that the blood samples taken from me are a gift and may be stored and used for the purpose of further research at a later date. I understand that these results will also remain anonymous.	☐
6. I understand that (this project or future research) will include genetic research aimed at understanding the genetic influences behind the development of osteonecrosis of the jaws in individuals taking bisphosphonates.	☐
7. I agree to take part in the above study	☐

Name of patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

UCL HOSPITALS

UCL Hospitals is an NHS Foundation Trust incorporating the Eastman Dental Hospital, Elizabeth Garrett Anderson & Obstetric Hospital, The Heart Hospital, Hospital for Tropical Diseases, National Hospital for Neurology & Neurosurgery, The Royal London Homoeopathic Hospital and University College Hospital.



8.3 CRF

CASE REPORT FORM

Bisphosphonate-Related Osteonecrosis Of The Jaws: A Case-Control Genetic Study

Chief Investigator: Prof. Stephen Porter
Principal Investigator: Dr. Stefano Fedele
University College London
Eastman Dental Institute
256 Gray's Inn Road
WC1X 8LD
London, UK

Version 2

1st June 2010

Date ____/____/_____ Investigator's Signature:_____

INDEX

1. Demographics

Patient ID [ ] [ ] [ ] Patient Study Number [ ] [ ] [ ]

Date of Birth: ...../...../.... Age: ……..

Gender [M] [F]

Ethnic Group: Caucasian [ ]

Black [ ]

Oriental [ ]

Other (please specify) [ ]

Vital signs: Pulse (bpm) ……..

Blood Pressure (mmHg): ……/…

Social History: Smoking Yes/No

Cigarettes [ ] cigars [ ] pipe [ ]

Alcohol (Units/week) ……

Date of enrollment: _____ / _____/ _____

2. Medical History

Please specify the reason for bisphosphonate therapy

	Diagnosis	Date of diagnosis (month/year)
[ ]	Metastatic cancer. Please define the primary site:	[][]/[][][][]
[ ]	Multiple Myeloma	[][]/[][][][]
[ ]	Hypercalcemia of malignancy	[][]/[][][][]
[ ]	Osteoporosis	[][]/[][][][]

Please list relevant secondary diagnoses
(Ask specifically for episodes of thrombosis and/or ischaemic disease)

No.	Diagnosis	Date resolved (month/year)	Tick if ongoing
1		[][]/[][][][]	[]
2		[][]/[][][][]	[]
3		[][]/[][][][]	[]
4		[][]/[][][][]	[]
5		[][]/[][][][]	[]
6		[][]/[][][][]	[]
7		[][]/[][][][]	[]

3. Drug History (General)

Medication	Date started (month/year)	Date stopped (month/year)	Tick if ongoing
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]

4. Drug History (Related to Bisphosphonates)

List details of INTRAVENOUS BISPHOSPHONATE THERAPY

Bisphosphonate Medication	Date started (month/year)	Date stopped (month/year)	Tick if ongoing
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]

List details of ORAL BISPHOSPHONATE THERAPY

Bisphosphonate Medication	Date started (month/year)	Date stopped (month/year)	Tick if ongoing
	[][]/[][][][]	[][]/[][][][]	[]
	[][]/[][][][]	[][]/[][][][]	[]

Please list adverse side effects of Bisphosphonate therapy

Side effect	Yes	No
Oral/oesophageal ulceration (for oral BP only)		
Bone pain		
Renal toxicity		
Hypocalcemia		
Flu-like symptoms		
Gastric upset		
Other: (please describe).....		

5. Inclusion criteria

Inclusion criteria differ between cases and controls.

5.1 Inclusion criteria for Cases* (Individuals on bisphosphonate therapy and with osteonecrosis of the jaws).

All items must be checked "yes" for the patient to be eligible for entry into the study

No.		Yes	No
1	Patient is on bisphosphonate medications		
2	Patient aged over 18 years		
3	Patient is capable of understanding the purpose of the trial and has given informed consent		
4	Patient has osteonecrosis of the jawbones diagnosed on the basis o currently accepted criteria *		

5.2 Inclusion criteria for Control Group (Individuals on bisphosphonate therapy without osteonecrosis of the jaws).

All items must be checked "yes" for the patient to be eligible for entry into the study

No.		Yes	No
1	Patient is on bisphosphonate medications		
2	Patient aged over 18 years		
3	Patient is capable of understanding the purpose of the trial and has given informed consent		
4	Patient has NO signs/symptoms of osteonecrosis of the jawbones diagnosed on the basis o currently accepted criteria.*		

* Diagnostic criteria for BOJ		Yes	No
1	Patient on BP therapy (and no history of radiotherapy of H&N)		
2	Chronic non-healing exposure of one or more areas of the jawbones through the oral cavity and/or facial skin (> than 8 weeks)		

3	Chronic pain, infection, purulent discharge, abscess, fistulas possibly associated		
4	Histopathological examination (when available) showing the presence of necrotic avascular bone with inflammatory infiltrate, and excluding other potential causes of jaw-bone exposure (e.g. metastases, primary cancer of the bone).		
5	CT or MRI (when available) of the jawbones showing one of more of the following (i) osteolytic lesions, (ii) sclerotic regions, (iii) periostal bone proliferation, (iv) reduction of the marrow space, (v) sequestration		

P.S There is increasing evidence in the literature that bone-exposure may not be required to diagnose ONJ (**non-exposed variant of ONJ**) . The **non-exposed variant of ONJ** presents with unexplained (not caused by dental or other bone disease)

- jaw bone pain
- fistula tract
- bone/gingival swelling

all in absence of frank transmucosal or transcutanoues bone exposure.

Clinical investigators are allowed to enroll these patients into the study but are required to highlight clearly that the patients has the non-exposed variant of ONJ

6. Exclusion criteria

Exclusion criteria differ between cases, control group A and control group B.

6.1 Exclusion criteria for Cases* (Individuals on bisphosphonate therapy and with osteonecrosis of the jaws).

All items must be checked "no" for the patient to be eligible for entry into the study

No.		Yes	No
1	Radiotherapy of the H & N		
2	Patient aged less than 18 years		
3	Patient is not capable of understanding the purpose of the trial and has not given informed consent		
4	Patient has no osteonecrosis of the jawbones diagnosed on the basis o currently accepted criteria *		

6.2 Exclusion criteria for Control Group (Individuals on bisphosphonate therapy without osteonecrosis of the jaws).

All items must be checked "no" for the patient to be eligible for entry into the study

No.		Yes	No
1	Patient is not on bisphosphonate medications		
2	Patient aged less 18 years		
3	Patient is not capable of understanding the purpose of the trial and has given informed consent		
4	Patient has signs/symptoms of osteonecrosis of the jawbones diagnosed on the basis o currently accepted criteria.*		

*** Diagnostic criteria for BOJ**

		Yes	No
1	Patient on BP therapy (and no history of radiotherapy of H&N)		

2	Chronic non-healing exposure of one or more areas of the jawbones through the oral cavity and/or facial skin (> than 8 weeks)		
3	Chronic pain, infection, purulent discharge, abscess, fistulas possibly associated		
4	Histopathological examination (when available) showing the presence of necrotic avascular bone with inflammatory infiltrate, and excluding other potential causes of jaw-bone exposure (e.g. metastases, primary cancer of the bone).		
5	CT or MRI (when available) of the jawbones showing one of more of the following (i) osteolytic lesions, (ii) sclerotic regions, (iii) periostal bone proliferation, (iv) reduction of the marrow space, (v) sequestration		

P.S There is increasing evidence in the literature that bone-exposure may not be required to diagnose ONJ (**non-exposed variant of ONJ**) . The **non-exposed variant of ONJ** presents with unexplained (not caused by dental or other bone disease)

- jaw bone pain
- fistula tract
- bone/gingival swelling

all in absence of frank transmucosal or transcutanoues bone exposure.

Clinical investigators are allowed to enroll these patients into the study but are required to highlight clearly that the patients has the non-exposed variant of ONJ

7. Date of diagnosis of osteonecrosis & local trigger and risk factors

7.1 Please detail the date of diagnosis of osteonecrosis (Month/Year)

[ ][ ]/[ ][ ][ ][ ]

7.2 Please detail the histopathology report (where available)

.....

.....

7.3 Please list the trigger/risk factor of osteonecrosis (if present)

Factor	***Date (Month/Year)***
Surgery to the jawbones (please detail):.....	[ ][ ]/[ ][ ][ ][ ]
Bone infection (Periodontal Disease)	[ ][ ]/[ ][ ][ ][ ]
Periapical infection (Apical Periodontitis)	[ ][ ]/[ ][ ][ ][ ]
Denture	Yes [ ] No [ ]
Tori	Yes [ ] No [ ]

8. Clinical examination

Extra-oral

Describe any swelling or fistula of the facial area

Area	Swelling	Fistulas
Right upper face	Yes [ ] No [ ]	Yes [ ] No [ ]
Right lower face	Yes [ ] No [ ]	Yes [ ] No [ ]
Left upper face	Yes [ ] No [ ]	Yes [ ] No [ ]
Left lower face	Yes [ ] No [ ]	Yes [ ] No [ ]

Intra-oral

Mandible

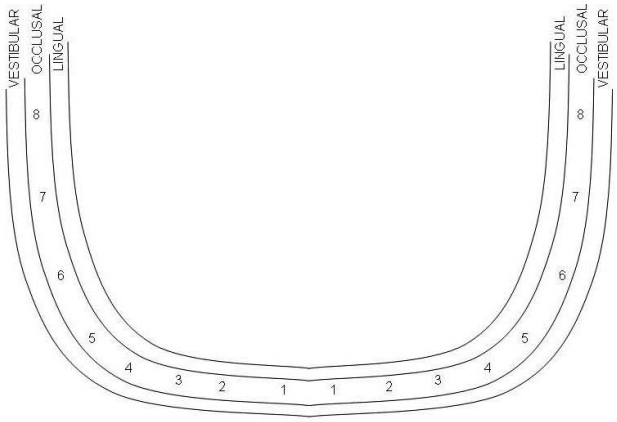
Please list and describe the area or areas affected and the dimension of the exposed bone area

Mandibular area	Vestibular	Occlusal	Lingual	Dimension (cm)	Other signs		
					swelling	suppuration	bleeding
Right (1)							
Right (2)							
Right (3)							
Left (1)							
Left (2)							
Left (3)							

Patient Initials: ____ Protocol Perio-09-41 **Subject # :___-___**

Bisphosphonate-Related Osteonecrosis Of The Jaws: A Case-Control Genetic Study

Please circle on the picture below the site(s) of involvement:



Maxilla

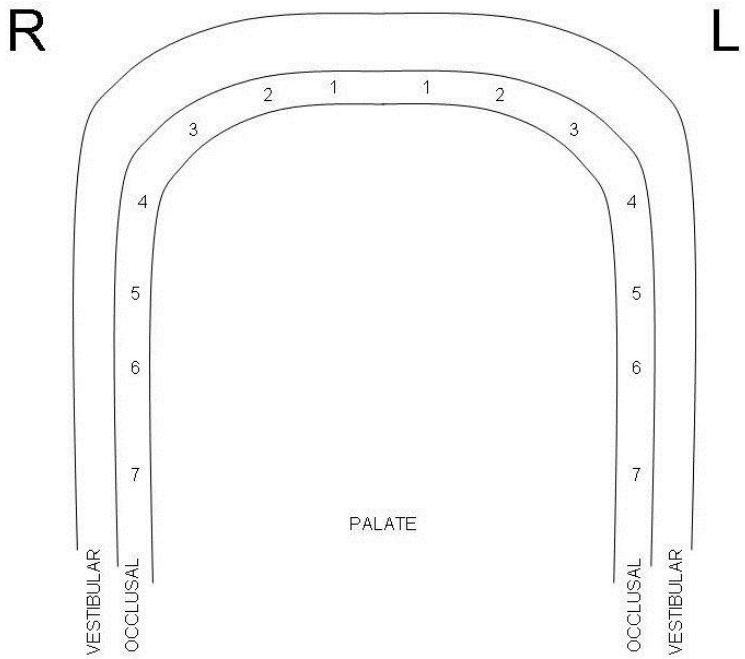
Please list and describe the area or areas affected and the dimension of the exposed bone area

Mandibular area	Vestibular	Occlusal	Palatal	Dimension (cm)	Other signs		
					swelling	suppuration	bleeding
Right (1)							
Right (2)							
Right (3)							
Left (1)							
Left (2)							
Left (3)							

Patient Initials:____ Protocol Perio-09-41 **Subject # :___-___**

Bisphosphonate-Related Osteonecrosis Of The Jaws: A Case-Control Genetic Study

Please circle on the picture below the site(s) of involvement:



9. Imaging techniques

Please detail the report of available OPT(s)

No	Date	Report

Please detail the report of available CT scan(s)

No	Date	Report

Please detail the report of MRI scan(s)

No	Date	Report

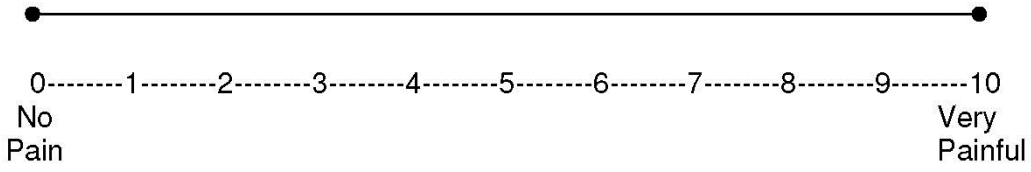
Please detail the report of Scintigraphy scan(s)

No	Date	Report

10. Symptoms

Pain [Yes] [No]

Intensity: VAS scale (0-10)



Other symptoms (paraesthesia, numbess etc).

Please detail:

.....

.....

.....

11. Blood sample

Date: ……../……../…….

mL of blood: ……..

Number of vials:

Code* on the vials:

* Please follow the following process for coding the vials:

BP- Centre Acronym-Patient's Initials-Patient's Study Number-Type of vial

For example, for a citrate vial from Mr John Smith, patient n.3 of the study attending the Eastman Dental Hospital (EDI), the code is:

BP-EDI-JS-003-Citrate

Termination Form

❑ Subject completed study

❑ Subject discontinued early

Please indicate the reason and explain in the comment section:

- ❑ Adverse event
- ❑ Unrelated illness
- ❑ Non-clinical or non-drug related reason
- ❑ Poor compliance
- ❑ Lost during follow-up
- ❑ Other

Comments:

_____

_____

_____

_____

Date ____/____/_____ Investigator's Signature:_____

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