Katholieke Universiteit Leuven
Group Biomedical Sciences
Faculty of Medicine
Department of Dentistry, Oral Pathology & Maxillo-Facial Surgery
Oral Imaging Center

# Validation and Diagnostic Performance of Jaw Bone Densitometry

Olivia NACKAERTS

Jury:

Promoter: Professor Dr. Reinhilde Jacobs
Copromoter: Professor Dr. Keith Horner
Chair: Professor Dr. Frans Vinckier
Secretary: Professor Dr. Joke Duyck
Jurymembers: Professor Dr. Paulo Couto Souza
Professor Dr. Hugh Devlin
Professor Dr. Ivo Lambrichts
Professor Dr. Frank Luyten

Leuven, 28.04.2009

Doctoral thesis in Medical Sciences

# Acknowledgements

A spring setting after a tough winter: the ideal moment to daydream and reminisce on the past years and all those who contributed to this work.

An inexpressible gratitude goes to my promoter, Reinhilde Jacobs. We met more than ten years ago, when she transferred the research bug to me. I want to thank her for believing from the very start that I could achieve this work. Her sincere support and friendship often gave me the confidence to take another step forward. I believe that not one person is more altruistic than her, always making time to listen, to help wherever possible and to share joyful and sad moments. My co-promoter, Keith Horner, has been a great scientific and linguistic advisor from overseas and was always there to set an optimistic tone. It has been such a pleasure and honour working with him, highly respected but always modest.

From the members of the thesis committee, I appreciated not only their valuable comments after revising the manuscript, but also their advice and considerations while proceeding with the studies and the manuscript. Thank you, professor Couto Souza, professor Devlin, professor Duyck, professor Lambrichts and professor Luyten.

Working on the Osteodent project has been an enriching experience, both on a scientific and on a personal level. I feel lucky to have been part of this warm group, of which many became friends for life. The actualisation of the Osteodent studies was only possible through the enormous efforts from the Manchester team, for which I want to thank them once more. Keith and Hugh, I will never forget the warm welcome into the project you gave me and your cordial support.

So many people stepped along with me for a while during the past years, some prominent, some behind the scene. I cannot possibly mention all of them and therefore I want to express a general word of appreciation to all those crossing my path, having influenced my way of thinking and having enriched my life. Mr. Beckers from Unident helped me developing the dedicated position holders, and I enjoyed very much our creative discussions. I want to acknowledge the lab for skeletal development and joint disorders under the guidance of professor Luyten, especially Jenny Peeters for her help in getting the first studies started. I also owe a lot to professor Boonen and Herman Borghs for making it possible to perform part of the clinical study at the centre for metabolic bone diseases. I am grateful

that professor Quirynen and professor Teughels gave me the opportunity to collaborate in research. I must truly thank Ivo, for his support in the last phase of the thesis, and for the help of his co-workers at the department of morphology, Hasselt university. The students that were working with me made an indispensable contribution to this thesis.

Since I was a student, the department of periodontology has been my ‘home at the job’. I want to say a heartfelt thank you to all the co-workers of this deparment for the serious and sometimes less serious conversations in the coffee room. Frieda, Liene and Wim, you have been examples for me, each in your particular way. Kris Cochet and Miranda Marechal, warm thanks for your kind help throughout the years.

To my colleagues at experimental ORL, it was great working with you and so nice to receive your supporting mails from ‘de berg’.

It has been amazing to work at the oral imaging centre, with colleagues from all over the world, discussing research and culture or simply having a relaxing drink after work. Bart, Livia, Paulo, Pisha, Baz, Ruben, Maria Eugenia, Yan, Maryam, Guozhi, Sam, Roland, Carine: Thanks! I want to express my gratitude more explicitly to Anna Maria, who brightened my life and broadened my view. Lily, it was a unique experience to have you as a travel mate in this thesis-journey, certainly for the past year. I have truly appreciated deciding on shared deadlines, the mutual peptalk and refreshing chats.

De Almagirls, de ‘low or no stress events’, de Puntoclub, het skiteam, Unisono, mijn nichtjes, la banda, my lost traveler, lieve vrienden… bedankt om mijn leven op te fleuren, ik draag jullie allemaal in mijn hart!

Ik ben blij dat ik eindelijk mijn familie hartelijk kan danken voor hun interesse en vele aanmoedigingen door de jaren heen. Guust en Nele, Tim en Kim, jullie steun van bij het begin heeft me zoveel deugd gedaan. De moeilijke tijden ben ik vooral dankzij jullie heelhuids doorgekomen, bedankt voor de deur die altijd openstaat! Flor, Stella en Gitte, bij sommigen ook wel bekend als ‘mijn kindjes’, zijn de grootste schatten in mijn leven! Zij zijn mijn bron van energie en relativeringsvermogen. Mama en papa. Ik heb van jongs af aan mogen ervaren dat jullie huis een plaats is waar mensen graag aanmeren. De waarden van gastvrijheid en belangeloosheid worden zeldzaam en ik ben zo fier dat ik ze van jullie meekreeg. Bedankt! Ik draag mijn thesis op aan jullie.

# Table of Contents

# Preface

This thesis is based on the following papers:

- Chapter 2

Nackaerts O, Gijbels F, Sanna AM, Jacobs R. Is there a relation between local bone quality as assessed on panoramic radiographs and alveolar bone level? Clin Oral Investig 2008;12:31-35

- Chapter 3

Nackaerts O, Jacobs R, Horner K, Zhao F, Lindh C, Karayianni K, van der Stelt P, Pavitt S, Devlin H. Bone density measurements in intra-oral radiographs. Clin Oral Investig 2007;11:225-229

- Chapter 4

Nackaerts O, Jacobs R, Pillen M, Engelen L, Gijbels F, Devlin H, Lindh C, Nicopoulou-Karayianni K, van der Stelt P, Pavitt S, Horner K. Accuracy and precision of a densitometric tool for jaw bone. Dentomaxillofac Radiol 2006;35:244-248

- Chapter 5

Nackaerts O, Jacobs R, Devlin H, Pavitt S, Bleyen E, Yan B, Borghs H, Lindh C, Karayianni K, van der Stelt P, Marjanovic E, Adams JE, Horner K. Osteoporosis detection using intra-oral densitometry. Dentomaxillofac Radiol 2008;37:282-287

- Chapter 6

Nackaerts O, Jacobs R, Devlin H, Horner K, Karayianni K, Mitsea A, Berkas L, Mastoris M, Lindh C, van der Stelt P, Marjanovic E, Adams J, Pavitt S. Is self-reported alcohol consumption associated with osteoporotic mandibular bone loss in women? Eur J Oral Sci 2009;117:7-12

- Chapter 7

Nackaerts O, Jacobs R, Quirynen M, Rober M, Sun Y, Teughels W. Replacement therapy for periodontitis: pilot radiographic evaluation in a dog model. J Clin Periodontol 2008;35:1048-1052

- Chapter 8

Nackaerts O, Couto Souza P, Devlin H, Horner K, Lambrichts I, Jacobs R. Density and structure of the jaw bone assessed in 2D and 3D radiography. (Submitted)

# List of abbreviations

| | |
|---|---|
| AED | Aluminium equivalent density |
| Al | Aluminium |
| BMD | Bone Mineral Density |
| BQI | Bone Quality Index |
| Ca | Calcium |
| CBCT | Cone beam computed tomography |
| CEJ | Cemento Enamel Junction |
| CPR | Clinical Prediction Rules |
| CW | Cortical Width |
| DXA | Dual energy X-ray absorptiometry |
| FD | Fractal Dimension |
| HU | Hounsfield Unit |
| MCI | Mandibular Cortical Index |
| OPG | Osteoprotegerin |
| ROC | Receiver Operating Characteristic |
| $A_z$ | Area under the ROC curve |
| ROI | Region Of Interest |

# Chapter 1

## General introduction and aims

## RADIOGRAPHIC JAW BONE ASSESSMENT

The assessment of jaw bone quality has a broad field of potential applications, such as preoperative planning of implant placement and the follow-up of bone changes as a result of disease progress or therapy. The assessment methods vary from subjective ordinal classifications to mathematical processing of the radiographic material (Jacobs et al 1996, Law et al 1996, Geraets & van der Stelt 2000, Jonasson 2005). A recurrent obstacle in studying these techniques is the acquisition of standardised images. More than in other skeletal sites, this standardisation is hard to achieve considering the complex jaw bone anatomy. Follow-up studies that require exact repositioning are hampered by this lack of standardisation as well as by potential changes that may occur intra-orally (tooth migration, attrition, extraction, bone loss …). Clinical tools for objective quantification of jaw bone quality are sparse. Studies on this often use image registration methods that are not easily accessible in most patient care facilities or are not validated (Ribeiro-Rotta et al 2007).

In addition, bone quality is often of critical importance in oral pathology and maxillofacial surgery. Bone healing can be hampered in poor bone quality which may be prone to rapid bone loss and even tooth loss (Bryant 1998, Lerner 2006). Furthermore, successful oral implant placement and osseointegration are to a large extent determined by the quantity (width and height) but also by the quality of the available jaw bone (Herrmann et al 2005, Jemt & Lekholm 1995). Therefore, the availability of bone quality data prior to surgery should therefore be considered a prerequisite as such to adapt surgical procedures, alter implant sites and types to avoid surgical complications and/or to enhance implant outcome.

For bone quality evaluation, no readily available and clinically reliable tools are present for routine use. Indeed, radiology is still to a large extent based on visual interpretation of radiographs, but the reliability of this interpretation has often been questioned (Ribeiro-Rotta et al 2007). Therefore, assessement of bone quality and its importance in therapeutical planning and monitoring the progress of local or systemic bone disease, will require more sophisticated methods.

## *Clinical methods in jaw bone assessment*

Several methods are used in research for jaw bone quality assessment, often in the pre-operative planning of implant placement. Since this work was oriented towards a tool for routine application, we focused on imaging methods that are present in a large proportion of dental offices or at least easily accessible for most dentists. These are panoramic and intra-oral radiographs, and, more recently, cone-beam CT (Gijbels et al 2005, Guerrero et al 2006).

## *2D radiography*

## Ordinal classification

Several classifications based on oral radiographs are used in research and clinical practice. These classifications enable quantification, albeit simple, of otherwise subjective observation of the radiographic characteristics of the jaw bones.

The Mandibular Cortical Index (MCI) subdivides the mandibular inferior cortex distally from the mental foramen into 3 classes: normal cortex (C1), mildly to moderately eroded cortex (C2) and severely eroded cortex (C3) (Klemetti 1993; Figure 1.1). The MCI is often used in research on osteoporosis screening (Taguchi 2008, Horner et al 2007).

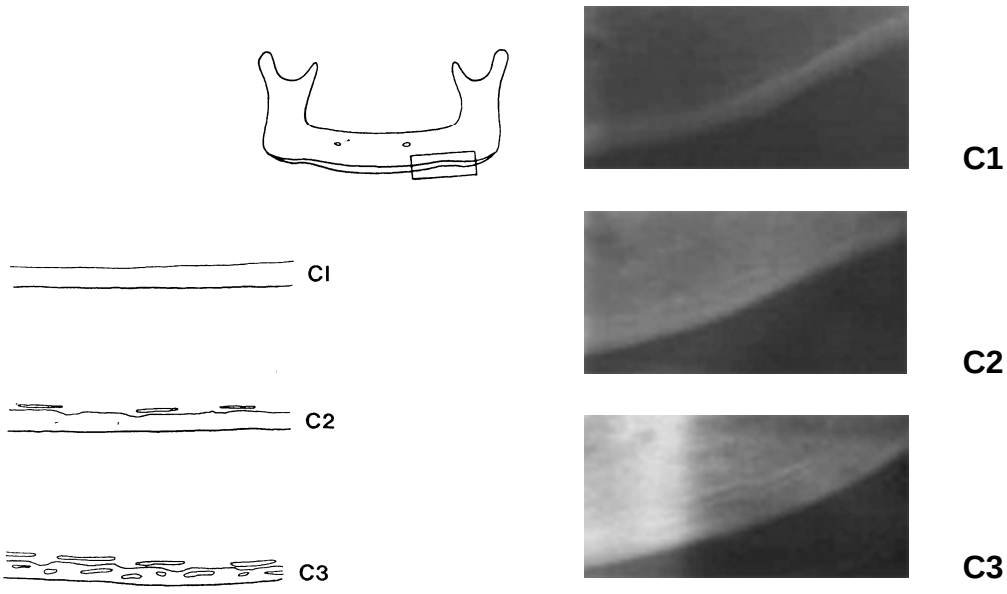



**Figure 1.1:** Mandibular Cortical Index and panoramic radiograph detail for each category (Klemetti 1993)

The Bone Quality Index (BQI) is often used in implant planning. Lekholm & Zarb (1985) developed the index as a predictive tool for implant success. Although it was intended for use in tomographic images, the BQI is nowadays mostly based on panoramic radiographs. There are 4 classes: homogeneous cortical bone (1), thick cortical bone with marrow cavity (2), thin cortical bone with dense trabecular bone of good strength (3) and very thin cortical bone with low density trabecular bone of poor strength (4) (Figure 1.2).

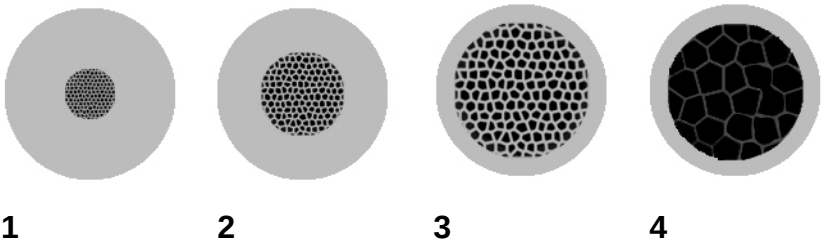



**Figure 1.2:** Bone Quality Index (Lekholm & Zarb 1985)

Lindh et al (1996) developed a classification of the trabecular pattern in intra-oral radiographs, classifying the pattern into: dense and homogeneous (A), heterogeneous (B) and sparse homogeneous (C). When performing the classification, reference radiographs are used to enhance interrater agreement (Figure 1.3). The Lindh classification is used in the assessment of bone quality before endosseous implant treatment and in research on osteoporosis screening.

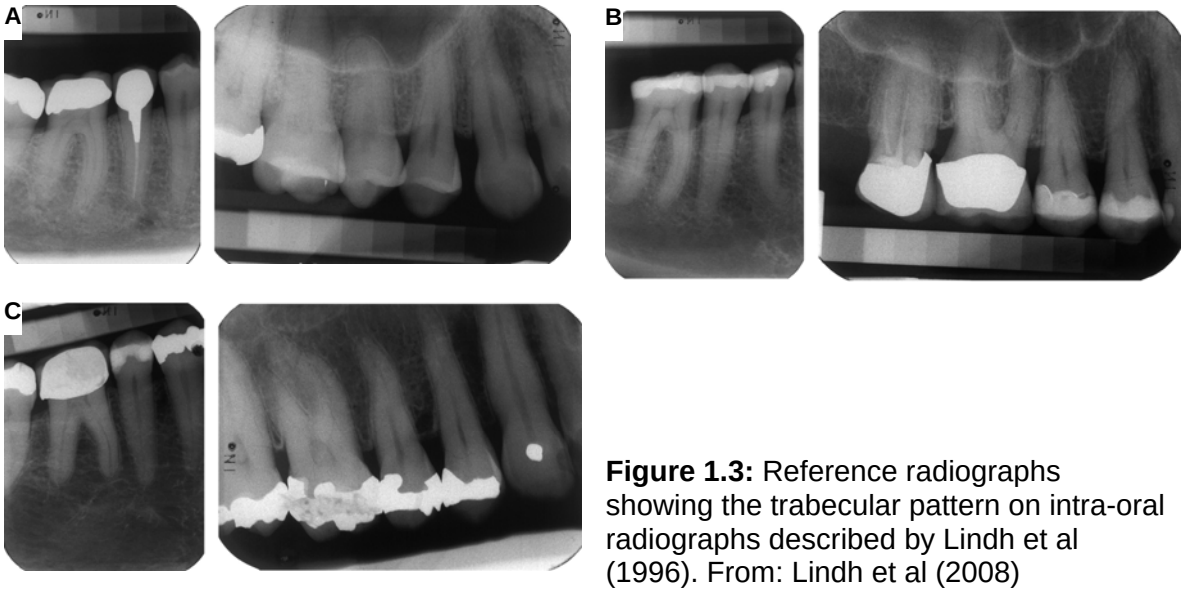



**Figure 1.3:** Reference radiographs showing the trabecular pattern on intra-oral radiographs described by Lindh et al (1996). From: Lindh et al (2008)

In these ordinal classifications, good inter- and intrarater agreement seems to be tricky, so whenever they are used in research, several observers should be included, as well as a training session to calibrate the observers. Moreover, it is not evident to find studies assessing the diagnostic accuracy of such classifications that fulfill the methodological criteria for diagnostic accuracy studies. Examples of problem issues in this context are the presentation of results that do not allow the actual assessment of diagnostic accuracy or biased interpretation of test results (Ribeiro-Rotta et al 2007). Although truly interesting from a clinical point of view, validation studies are needed to determine the true relevance in clinical practice of these classifications.

## Radiographic density

The degree of darkening of an image receptor exposed by X-rays is referred to as radiographic density. The radiographic density is influenced by exposure and thickness and density of a subject (White & Pharoah 2004).

In the assessment of bone quality, the mean and standard deviation of grey values in the region of interest can be used. However, grey values have limited use, as they can be influenced by many other factors, apart from actual bone changes or characteristics.

To obtain a more meaningful result, many researchers have used subtraction radiography for bone density changes on follow up radiographs. Although a promising technique if applied with high precision (Christgau et al 1998, Rawlinson et al 1999, Du Tré et al 2006), digital subtraction is hardly feasible in clinical studies. The accurate interpretation of subtraction images demands pairs of radiographs of close to identical x-ray beam projection geometry, film position and contrast (Hausmann 2000). Digital image subtraction may yield quantitative, albeit relative, results, expressed as ‘number of changed pixels x mean grey level change’, when using computer assisted densitometric image analysis (CADIA) (Brägger 1988). Although a step forward towards quantification of bone densitometric analysis, the comparison of change at different regions of interest requires a mineral or aluminium stepwedge for internal references (Matteson et al 1996). An aluminium stepwedge has similar absorption and scatter properties of bone (Trouerbach 1984) and is therefore suitable for densitometric analysis and follow-up (Jacobs et al 1996) (Figure 1.4). Other materials suggested are

hydroxyapatite and barium sulfate (Yang et al 2002) and nickel (Horner & Devlin 1998).

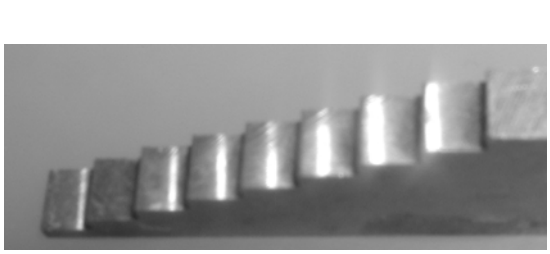
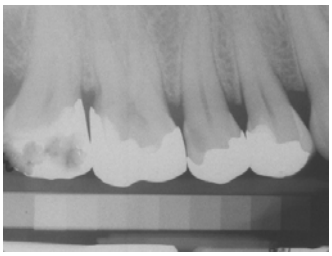

**a**

**b**

**Figure 1.4:** a) Aluminium step wedge; b) Step wedge integrated in periapical radiograph

## Radiomorphometry

Radiomorphometry is the application of anthropologic indices of bone morphology to radiographs. Mostly, these indices are based on measurements of cortical bone because it is more distinct on radiographs than trabecular bone.

*Panoramic Mandibular Index (PMI)*

The PMI was initially developed as a tool for osteoporosis screening by dentists, using a relative measure for cortical thickness (Benson et al, 1991). First, the shortest distance between the inferior border of the mandible and the superior and inferior margins of the mental foramen on panoramic radiographs is determined. On this line, the cortical width is measured and PMI is calculated as:

PMI (superior) = Thickness of cortex/Distance from superior margin of mental foramen to inferior border of mandible

PMI (inferior) = Thickness of cortex/Distance from inferior margin of mental foramen to inferior border of mandible.

*Mandibular cortical thickness*

In several studies, the PMI showed poor interobserver agreement (Ledgerton et al 1997, Horner & Devlin 1998). This is not surprising, since the location of the mental foramen on a panoramic radiograph is not always unequivocal, let alone the definition of the upper and lower border. Furthermore, the index is based on two linear measurements, which introduces a cumulative error in the measurement. At present, the mandibular cortical thickness is more often used as a morphometric jaw bone index on panoramic radiographs. To determine the width, first a tangent to the lower mandibular border is drawn. Through the mental foramen, a perpendicular line to the first is created. On this perpendicular line, the

cortical width is measured (Figure 1.5). If the cortex shows erosion, the thinnest compact cortical width should be considered.

*Residual ridge height*

Besides absolute and relative measures of local alveolar bone height in the evaluation of periodontal bone loss, residual ridge height is used to assess generalised ridge resorption (Xie et al 1997). It is usually measured at the mental foramen site. It is also based on the tangent to the inferior mandibular border. The perpendicular to this tangent intersecting the inferior border of the mental foramen is the line used for measuring the ridge height; it is measured from the lower border of the mandible to the top of the alveolar crest.

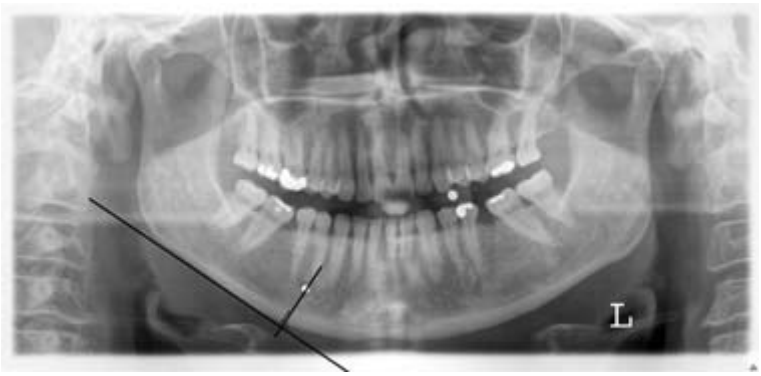



**Figure 1.5:** Mandibular cortical width measurement on a panoramic radiograph (cfr Chapter 6)

## Texture analysis

Texture analysis of trabecular bone requires digital manipulation of the images. Due to the application of mathematical operations on the image, the results often depend on the applied parameters, but the analysing methods are more robust in quantifying the images than all previously mentioned methods (Apostol et al 2006). This often makes textural analysis the method of choice in imaging research. A vast number of texture parameters exist; the ones described below do not form an exhaustive list, but are a selection of frequently used parameters in describing jaw bone structural properties.

The processing of 2D radiographs for texture analysis of bone starts with a binarisation of the image or the region of interest (Figure 1.6). The bone is segmented as the foreground signal, often following an initial filtering to remove noise from the image. The segmentation is usually done with a thresholding technique. A binarised image can be seen on Figure 1.6b. Based on the binary

image, the bone area can be calculated, as well as the background area. The number of black and/or white regions can be counted, to have an idea of connectivity of the bone. By further skeletonising of the binary image (Figure 1.6c), a number of measurements can be done on the wire version of the image. These are for example the total length of the frame, the number of endpoints and the number of nodes. This can be done for the white skeleton as well as the black.

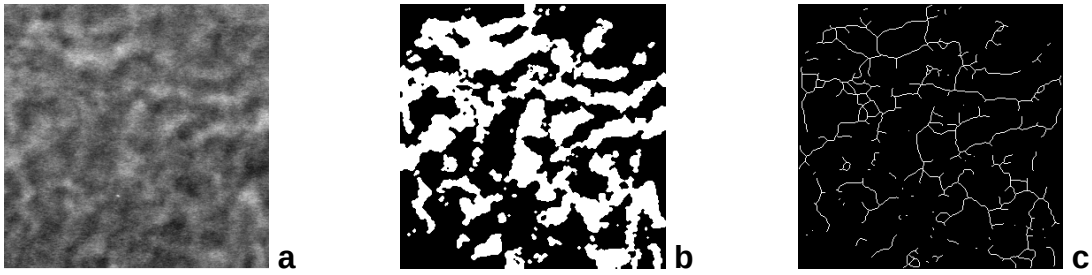



**Figure 1.6:** a) Region of interest from original radiograph; b) Binarised region of interest; c) Skeletonised region of interest

Fractal analysis is one of the textural analyses that is used for the characterisation of bone, but also the arteriovenous and neural architecture, the dynamics of the heartrate, pathology etc. A fractal is a complex geometric shape. Its main property is self-similarity: it can be split into parts, each of which is a reduced-size copy of the whole shape (Mandelbrot 1993). Fractal analysis is a method for describing such complex shapes and structural patterns, expressed numerically as fractal dimension (FD). There exists a variety of methods for calculating fractal dimension of bone (for review: Geraets & van der Stelt 2000), of which the box counting algorithm is often applied. The region of interest is covered with boxes of increasing size and the number of boxes covering the actual pattern is calculated. The results of this algorithm are plotted on a log-log plot. The slope of the resulting line is the FD + 1.

## *Cone beam CT (CBCT) imaging*

Compared to 2D radiographic methods, 3D imaging provides better diagnostic accuracy and a more efficient surgical planning for dental applications, such as implant planning, endodontic treatment and tooth impaction (Guerrero et al 2006, Patel et al 2007, Neugebauer et al 2008). For decades, spiral CT was the modality of choice when 3D imaging of the jaw was required. Notwithstanding the diagnostic and treatment benefits, the main drawback of conventional CT is the

high radiation dose from this technique. CBCT has been more recently developed and implemented in disciplines like cardiology, radiotherapy and dentistry and it overcomes to a large extent the radiation dose issue (Guerrero et al 2006). A CBCT system allows the acquisition of 3D data in only one rotation. Still, spatial resolution can reach levels up to 75 µm (Iluma® ultra cone beam scanner, Kodak, Rochester, NY-USA). The lower radiation dose and the cone shape of the X-ray beam is associated with a higher amount of image noise and artefacts compared to conventional CT. Because of the CBCT characteristics, Hounsfield units, the standard in bone density evaluation on CT images, are not applicable. Since images of high resolution can be produced of the jaw bone, without the need for biopsies as would be the case for µCT or histology, it is worthwhile investigating methods for objectively quantifying bone quality on CBCT images (Yan et al 2009).

To analyse the bone structure, 3D characteristics as in µCT can be applied. After segmentation, morphological measures like trabecular number, trabecular separation, trabecular thickness and bone volume relative to total volume can be calculated.

## JAW BONE AND SYSTEMIC BONE

### *Osteoporosis*

Osteoporosis is a systemic bone disease and one of the most prevalent diseases in the elderly. The pathogenesis of osteoporosis is complex and multifactorial. Due to an unbalanced bone remodelling cycle, more bone is resorbed than newly formed, causing a lower bone mineral density and a deterioration of bone microarchitecture, leading to a higher susceptibility to fractures. Because of the ageing population and a qualitative deterioration of food and lifestyle factors, the prevalence of osteoporosis is believed to steadily increase. Moreover, the patient population developing secondary osteoporosis caused by chronic medication is rising. After the occurence of a fracture, the chance of subsequent trauma rises and so does morbidity and mortality (Haentjens et al 2003). To be able to stop the societal cost associated with osteoporosis, it is of critical importance to identify these patients at an early stage and start preventive treatment. Although bone mineral density as measured by

dual energy X-ray absorptiometry (DXA) is a very strong predictor of bone strength, there are other determinants, such as trabecular connectivity, cortical bone size and skeletal morphometry (Rosen 2006). Still, BMD represents the easiest parameter for risk assessment. DXA machines are widely available but too expensive for population based screening. Alternative tests are available but access to these tests and the identification of patients at risk is still difficult.

## *Osteoporosis and the jaw bone*

Much research has been done on the detection of low systemic bone mineral density using oral radiographs. Dental practitioners regularly use panoramic and intra-oral radiographs for diagnostic purposes. As such they are readily available for the vast majority of patients, which makes these radiographs ideal for large-scale osteoporosis screening. It is not self-evident to perform such studies. Bone density differs depending on the region where it is assessed and this is certainly the case in the jaw bone. Maxillary bone consists mainly of trabecular bone with a large surface of blood supply. The mandible has a lower proportion of trabecular bone and is surrounded by a dense cortical border. Besides, jaws are influenced by local factors, related to the presence or absence of teeth, which are in direct contact with the environment. As long as the periodontium and teeth are present, these local influences can interact with systemic factors to affect jaw bone metabolism. In edentulous patients, the systemic influence gains importance in determining jaw bone properties (Slagter et al 2008).

It is accepted that the jaw bone of persons with osteoporosis has a lowered bone mass as compared to healthy individuals (von Wowern 1988, Kribbs et al 1990, Jacobs et al 1996). Morphometric indices on panoramic radiographs do not seem to have high enough sensitivity and specificity for osteoporosis detection (Devlin 2002, Dutra 2005, Devlin et al 2007c, Horner et al 2007). Visual assessment of the trabecular pattern in intra-oral radiographs could be useful to identify women at risk of having osteoporosis, but intrarater agreement remains an issue to be solved here (Lindh et al 2008). The mandibular cortical width is a successful screening tool for osteoporosis (Taguchi et al 1996, Nakamoto et al 2003), especially when combined with clinical risk indices such as age and BMI (Karayianni et al 2006, Devlin et al 2006, Devlin et al 2008). Another promising

screening tool is fractal dimension (Jonasson et al 2001, White et al 2005). Results in osteoporosis research that report fractal dimension are still inconsistent though, both in *in vivo* and *in vitro* research. In some publications, patients with osteoporosis show higher fractal dimension than controls and in others it is the reverse. Even in well-controlled *in vitro* studies, various studies yield different results. This is caused by alternate sites measured, diversity in imaging modalities and in methods for fractal analysis (van der Stelt 2000). To obtain more consistent results and to be able to draw definite conclusions, it will be important to specify all these factors in future research on the topic. Meanwhile, the prediction of BMD with other textural parameters described above is feasible (Geraets et al 2007).

## *Diagnostic tests*

A diagnostic test can be characterised by its sensitivity, specificity, predictive value and likelihood ratio. A short introduction into the matter of evaluation of diagnostic tests seems in place here (based on an extensive description by Bonnick, 2004). Sensitivity stands for the ability of a test to select diseased individuals out of a population. It is the proportion of diseased individuals who test positive to the total number of diseased individuals. Specificity is the proportion of non-diseased individuals who test negative to the total number of non-diseased individuals. Positive and negative predictive values stand for comparable proportions as sensitivity and specificity, but with the test results instead of the actual disease state as a denominator. Likelihood ratios are calculated using sensitivity and specificity of a test. Positive likelihood ratio is the ratio of the probability of a positive test in diseased patients to the probability of a positive test in patients who are not diseased: LR+ = Sensitivity/1-Specificity. Negative likelihood ratio is the ratio of the probability of a negative test in patients with the disease to the probability of a negative test in patients who are not diseased: LR- = 1-Sensitivity/Specificity.

Sensitivity and specificity of a diagnostic test are depending on the cut-off point chosen to identify a person as diseased or not. Changing a cut-off point to obtain higher sensitivity, will result in decreased specificity (more false positive results). The relationship between sensitivity and specificity can be examined using a ROC curve (Chapter 5).

# AIMS

The overal aim of this thesis was to develop and validate an objective, clinically applicable tool for jaw bone density evaluation.

Initially, panoramic radiographs were used in assessing jaw bone density and its predictive value for the degree of marginal bone loss. Facing the methodological constraints of using panoramic radiographs, such as the enlargement factor, spatial resolution and geometrical variability, intra-oral radiographs were adopted as the imaging tool of choice for developing our method: it has low radiation dose, high resolution, no or minor enlargement and also this type of radiograph is readily available for the majority of patients. CBCT is a recent development in dentomaxillofacial radiology, but its use is increasing (www.sedentexCT.eu). Although promising in diagnostic performance, there is no valid measure for bone density assessment, comparable to e.g. Hounsfield Units in multi-slice CT. As part of our future research perspectives, we applied a method for bone quality evaluation in CBCT images.

The specific aims of the present thesis were:

**Chapter 2:** To evaluate densitometry on panoramic radiographs as a determinant of periodontal bone loss.

Hypothesis: Locally diminished bone density aggravates periodontal bone loss.

**Chapter 3 & 4:** To develop and validate a densitometric tool for jaw bone on intra-oral radiographs *in vitro.*

**Chapter 3**

Hypothesis: Aluminium equivalent jaw bone density yields more accurate results than mere grey values.

**Chapter 4**

Hypothesis 1: Aluminium equivalent jaw bone density is a precise method for bone density assessment.

Hypothesis 2: Aluminium equivalent jaw bone density is an accurate predictor of bone mineral density.

**Chapter 5-7:** To validate the densitometric tool for jaw bone on intra-oral radiographs *in vivo.*

**Chapter 5**

To determine diagnostic accuracy of jaw bone densitometry for osteoporosis detection.

Hypothesis: Jaw bone density can predict osteoporosis.

**Chapter 6**

To determine the influence of alcohol and smoking on jaw bone density.

Hypothesis: Alcohol consumption has an influence on jaw bone density.

**Chapter 7**

To use jaw bone densitometry for periodontal defect follow-up in bacterial replacement therapy.

Hypothesis: Bone densitometry shows enhanced periodontal pocket healing by subgingival application of beneficial bacterial species.

**Chapter 8:** To evaluate the accuracy of jaw bone quality assessment on intra-oral radiographs and CBCT images.

Hypothesis: Densitometry and texture analysis are accuracte predictors of bone structure.

# Chapter 2

## Is there a relation between local bone quality as assessed on panoramic radiographs and alveolar bone level?

## INTRODUCTION

Oral radiographs are an important diagnostic tool in dental practice. Both teeth and surrounding structures can be inspected on intra-oral or extra-oral radiographs. When assessing jaw bone on oral radiographs, both bone quality and bone quantity can be evaluated. Assessment of jaw bone quantity is performed on a daily basis when screening for or diagnosis of periodontitis (Goodson et al 1984, Hausmann et al 1991, Walsh et al 1997). Bone quality is most often evaluated in the periapical region when endodontic problems are encountered (Friedlander et al 2002, McCaul et al 2001). Overall jaw bone quality, however, is less often assessed, although it could be an important aid in diagnosis of bone diseases such as osteoporosis (Horner & Devlin 1992, Klemetti et al 1993, Nakamoto et al 2003, Taguchi et al 2004, Inagaki et al 2005, Lee et al 2005) or to identify individuals at higher risk of alveolar (periodontal) bone loss (Shen et al 2004). Bone quality differs between individuals (Nelson & Megyesi 2004) and can be assessed on different types of radiographs. Various approaches have been used to determine jaw bone quality.

On panoramic radiographs, a range of indices are described for jaw bone quality assessment. Morphologic indices are, for example, the Mandibular Cortical Index (MCI) (Klemetti et al 1994) and the Bone Quality Index (BQI) (Lekholm & Zarb 1985). The MCI describes the appearance of the lower mandibular cortex as smooth (C1), with semilunar erosions (C2) or as porous (C3). The BQI is a method to describe the bone quality depending on the amount and the proportion of cortical and trabecular bone. There are four classes: I, homogenous cortical bone; II, thick cortical bone with marrow cavity; III, thin cortical bone with dense trabecular bone of good strength; IV, very thin cortical bone with low density trabecular bone of poor strength. Other indices concentrate on bone density rather than bone morphology.

Research on the relation between osteoporosis and periodontal disease suggests a greater propensity to lose alveolar bone in subjects with osteoporosis (Elders et al 1992, Hildebolt et al 2002). In other words, osteoporosis, or low systemic bone mineral density (BMD), should be considered a risk factor for periodontal disease progression (Geurs et al 2003). However, there is a variety of confounding factors, such as age, genetics, bacterial infections, systemic disease,

stress, socio-economic status, oral hygiene and smoking (Persson 2006, Yoshihara et al 2004). Osteoporotic patients are shown to have a lower jaw BMD than controls (von Wowern et al 1994, Payne et al 1997, Civitelli et al 2002) and mandibular bone density has been shown to correlate with skeletal density (Takaishi et al 2004). In addition, film densitometry of mandibular bone is shown to correlate to vertebral densitometric data according to a number of studies (Kribbs 1990, Kribbs et al 1990, Law et al 1996). Other studies cannot confirm a clear correlation between the quality and/or quantity of oral and systemic bone (Mohajery et al 1992). When bone quality and bone quantity are analysed on oral radiographs, it should be kept in mind that besides osteoporosis, other diseases can affect jawbone characteristics. Tumours and cysts of the jaws and other bone diseases influence radiographic bone quality (Soikkonen et al 1994).

The aim of the present study was to investigate the relation between bone quality as seen on panoramic radiographs and loss of alveolar bone level. Bone quality was assessed by radiographic bone density and using the BQI. The hypothesis to be rejected was that there is no relation between local bone quality and the alveolar bone level.

## MATERIALS & METHODS

Digital panoramic radiographs of 94 female patients were assessed. Inclusion criteria were as follows:

1) Minimum age of 35 years;

2) Absence of considerable infection or bone pathology (cysts, tumours);

3) Presence of all premolars in the lower jaw and at least four teeth per quadrant;

4) Cemento-enamel junction (CEJ) clearly visible on both sides (no destruction due to caries or restorations).

All panoramic exposures were made with a Cranex TOME® (Soredex, Helsinki, Finland) multimodal machine. The panoramic radiographs were acquired with storage phosphor plates (MD10XHQ®, Agfa, Mortsel, Belgium) and read out in an ADC Solo® phosphor plate scanner (Agfa). The Agfa MUSICA® software was used to view the images and determine the density. No additional image enhancement

was applied apart from the standard software settings. All exposures were done by the same radiographer within a limited time period (consecutive patients).

The radiographic bone density, expressed in gray value, was determined in between the premolars in the mandible, at the lower third of the root (von Wowern 2001). A grid was placed on the panoramic radiograph, and the observers selected a square sized 2x2 mm (region of interest) from the grid.

The alveolar bone level was expressed as a percentage and calculated as follows: (A/B)*100 with “A” being the distance (in mm) from the CEJ to the alveolar crest. The measuring point at the alveolar crest was defined as the most coronal location of the bone margin adjacent to the ligament space. “B” stands for the distance (in mm) from the CEJ to the apex (Figure 2.1) (Jacobs & van Steenberhe 1998). Measurements were done between the premolars, more specifically distal from the first and mesial from the second premolar, as to be able to relate the data to the local bone density measurements. Results were averaged for analysis. The percentages expressed the loss of alveolar bone level, relative to the root length. Because of normal anatomical variance (White & Pharoah 2004), a loss up to 10% was not considered ‘bone loss’ for statistical analysis.

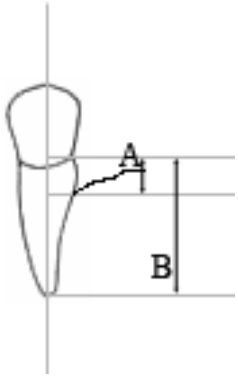



**Figure 2.1:** Alveolar bone level loss was calculated as (A/B)*100, with “A” being the distance from the CEJ to the alveolar crest and “B” being the distance from the CEJ to the root apex. The distance was measured on the lines perpendicular to the root axis, intersecting the points of interest: CEJ, alveolar crest and root apex (Jacobs & van Steenberghe 1998). The measuring point at the alveolar crest was defined as the most coronal location of the bone margin adjacent to the ligament space

The BQI was assessed (Lekholm & Zarb 1985). As this index is considered a rather robust way of evaluating bone quality (Lindh et al 1996) and this was observed in the data set, we opted for a binarisation of the data and recoding of two categories only: high bone quality, being the combination of quality 1 and 2 (1) and low bone quality, pooling quality 3 and 4 (2). Two observers did a test–retest assessment of the panoramic radiographs for all measurements.

### *Statistical analysis*

Statistical analysis was performed with Medcalc® vs 9.2.0.2. (Medcalc, Mariakerke, Belgium). Because data did not show normal distribution, Spearman's r was used to investigate the relation between bone density and alveolar bone level. For relating the BQI to the alveolar bone level, the Kruskal–Wallis test was performed. Interobserver repeatability was tested with the concordance correlation coefficient. This coefficient does not only take into account the deviation of each individual measurement but also the deviation of the regression line from the 45° line through the origin. The level of significance was set at $p = 0.05$.

## RESULTS

The patient characteristics of the 94 subjects are shown in Table 2.1. The age ranged from 35 to 74 yrs, with a median of 44 yrs.

**Table 2.1:** Patient characteristics

| | Median | Minimum | Maximum |
|---|---|---|---|
| **Age** | 43 | 35 | 74 |
| **Jaw bone density** | 545 | 415 | 764 |
| **Percentage loss of alveolar bone level** | 20.0 | 13.6 | 32.3 |
| **Number of teeth missing** | 8 | 4 | 15 |

Bone density in the lower jaw and the percentage loss of alveolar bone level were significantly correlated ($p < 0.05$). The Spearman's coefficient of rank correlation was –0.27. The scatterplot is shown in Figure 2.2. The Kruskal–Wallis test showed a tendency towards a significantly higher loss of alveolar bone level in category 2 of the simplified BQI (low quality). The concordance correlation for the intra- and interobserver agreement were 0.99 and 0.98, respectively, for the density evaluation and 0.91 and 0.90, respectively, for the bone level assessment.

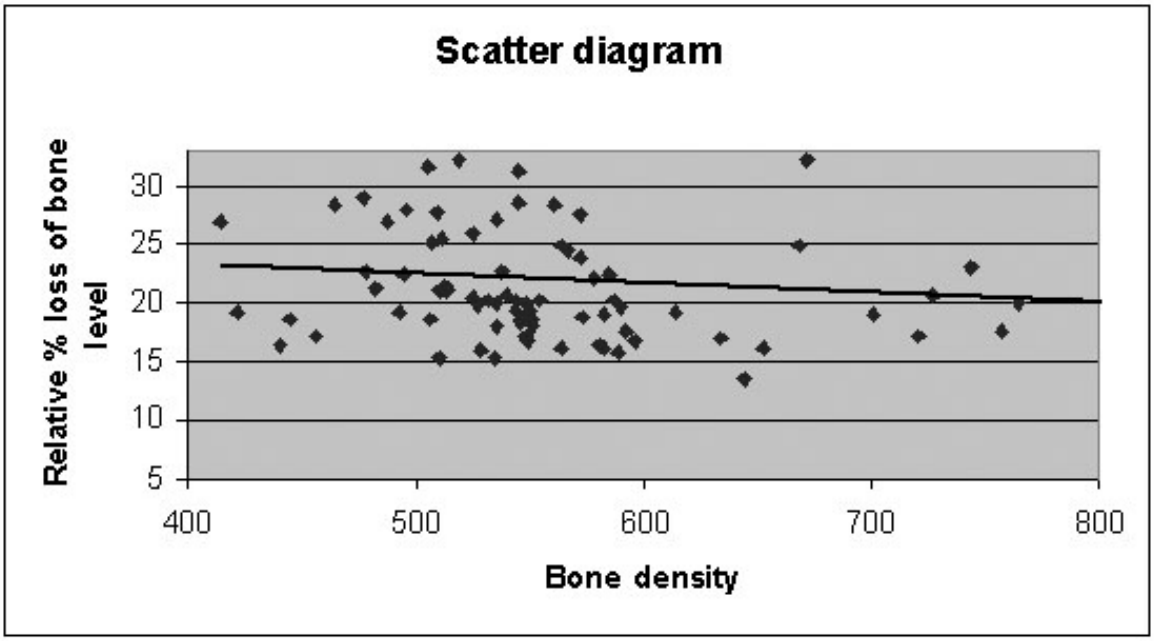



**Figure 2.2:** Scatter plot showing negative correlation between bone density and relative percentage loss of alveolar bone level

## DISCUSSION

There was a weak but significant relationship between mandibular radiographic bone density and the loss of alveolar bone level in the premolar area of the lower jaw. Previous studies suggested a relationship between alveolar bone level and skeletal bone density (Payne et al 1999, Mohammad et al 2003, Taguchi et al 2004, Yoshihara et al 2004, Takaishi et al 2005), but other studies did not find such a correlation (Lundström et al 2001, Famili et al 2005).

Possible explanations for the low correlation found between local bone density and jaw bone level in the current study were sought for. The first one could be the influence of confounding factors for which the data set was not controlled. Bone resorption is influenced by multiple factors. In edentulous areas, there is great interindividual variation for what concerns resorption rate. A single dominant factor for residual ridge resorption cannot be indicated, but factors considered of importance are gender, age, facial morphology, duration of edentulousness, oral hygiene, oral parafunctions, occlusal loading, nutriton, general health, medication, systemic diseases and osteoporosis (Carlsson 2004). The resulting resorption is a process involving both local and systemic factors, but it is likely to be more related to systemic factors (Devlin & Ferguson 1991). In the current study, the

measurements were done in a dentate area. In stead of edentulous bones, the local influence is in this case not related to denture wearing, but to other external factors such as periodontal infection. When the jaw bone density is low, e.g. due to post-menopausal osteoporosis, the progression of alveolar bone loss due to periodontal disease may be enhanced (Lerner UH 2006). Other factors that have a more or less established place in the prediction of the severity of periodontal bone loss are bacterial infections, restorations, periapical status, socio-economic status, oral hygiene and smoking (Nelson & Megyesi 2004, Müller et al 2005). Investigating the role of all of these falls out of the scope of the current study that evaluates the applicability of panoramic radiographs to detect the relation between loss of alveolar bone level and jaw bone quality. Due to the selection criteria, certain influencing factors were not possible to be evaluated: gender, since it was a female sample; major bone pathology, since subjects suffering from it were excluded. Although information on the subjects' age was collected, no significant correlation could be found between this information and the alveolar bone level. This is in contradiction with most research (Reddy & Jeffcoat 1993, Bahrami et al 2006), which is probably due to selecting subjects where 4 or more teeth per quadrant were present, excluding in such way subjects with low oral hygiene or severe periodontal pathology.

It might also be that the gray level of the images was not a valid measure for bone density. We investigated this, comparing bone density measurements with and without the inclusion of a reference wedge (Nackaerts et al 2007). Although the measurements without a reference wedge included showed some correlation towards actual density, the accuracy was far higher in the 'reference' measurements. Another study indeed confirmed the value of an aluminium wedge, e.g. for the detection of subtle changes in jaw bone density as opposed to merely gray scale correction (Du Tré et al 2006). To enable clarification, it would be necessary to perform a clinical study, including a reference stepwedge when the panoramic radiograph is taken.

Perhaps, the structure of the trabecular bone, rather than only the density, is a factor influencing loss of periodontal bone level (Landini 1997). Structural analysis such as fractal dimension or three-dimensional images would be better for characterising the trabecular bone (Geraets et al 2006; van Eijden et al 2006). The

chosen region of interest is most suitable for jaw bone density measurements because of its small intra- and inter-individual variability for what concerns anatomical size, shape, bone structure and function (von Wowern 2001).

It is clear that panoramic radiographs are very useful overview images, but for absolute linear measures, they are surely not the method of choice, unless the radiographs are standardised (Müller & Ulbrich 2005). That is why it was decided to express the loss of alveolar bone level as a ratio, relative to the root length. Test–retest reliability was good in assessing the bone level on panoramic radiographs.

The question arises whether it is sufficient to use a simple jaw bone density assessment to predict the future loss of alveolar bone level. Obviously, a prospective study design is needed to verify this hypothesis. Moreover, thought should be given on the best method to assess jaw bone in an uncomplicated, standardised way. A bone quality index might also be predictive of periodontal bone level loss if a more objective index could be developed. An attempt was made by Lindh et al (1996) to achieve such index, by including reference radiographs in the assessment protocol. Nevertheless, this scoring was based on intra-oral radiographs only, and it is up to now not investigated as a potential tool for panoramic radiography.

In conclusion, radiographic density of the local alveolar bone seems to influence periodontal bone level loss to some extent. If further studies are to be conducted in investigating local bone quality as a predictive factor for alveolar bone loss, it is advised to integrate structural analysis of the bone, e.g. the assessment of fractal properties or using three-dimensional imaging and to perform longitudinal research.

# Chapter 3

## Bone density measurements in intra-oral radiographs

## INTRODUCTION

Assessment of jaw bone density may be considered as useful or even necessary in many clinical situations to asses bone tissue. Applications include diagnostics of oral and/or systemic diseases, implant planning, therapeutic evaluation and follow-up. In this perspective, most research has been focused on the prediction of low skeletal bone density from oral radiographs (Dervis 2005, Law et al 1996, von Wowern 2001, White 2002). Jaw bone density assessment can be based on intra-oral radiographs (White et al 2005, Yang et al 2002), panoramic radiographs (Dural et al 2005, Knezovic-Ziataric & Celebic 2003), medical, cone beam and micro-computed tomography (Aranyarachkul et al 2005, Shapurian et al 2006, Stoppie et al 2006), DXA (Corten et al 1993, Devlin et al 1998), magnetic resonance imaging (Choel et al 2004) and quantitative ultrasound (QUS) (Nicholson et al 1996).

If research findings in this field are to be clinically applied, there is a need for a widespread, low cost, user- and patient-friendly tool for bone density evaluation. Furthermore, the tool needs to be accurate and the measurements precise and reproducible. As mentioned above, many techniques for jaw bone density measurements exist and are used clinically and/or experimentally (von Wowern 2001, White 2002). It is not an easy task to select from these the ultimate tool for large-scale jaw bone density analysis.

Intra-oral radiographs do live up to most requirements of the ideal tool. They are commonly used, at a low cost and easy to obtain. Various methods of analysis, both complex and simple, can be applied to intra-oral radiographs: fractal analysis (Geraets & van der Stelt 2000), the classification of the trabecular pattern (Lindh et al 1996), or densitometry (Jacobs et al 1996, Jonasson 2005). The focus of the current study is densitometry, which might be useful for osteoporosis screening, but also for bone site evaluation before implant placement and to evaluate therapy involving bone. To be able to obtain comparable results in densitometry, it is useful to include a reference material in the radiograph. The aluminium step wedge is frequently used in bone density research because the absorption and scatter properties are similar to those of bone (Trouerbach et al 1984). Therefore, a comparison can be made between the density produced on the radiograph by the wedge and that produced by the bone. To include a step

wedge on an intra-oral film is an additional burden for the dentist. To accommodate the step wedge, image space must be sacrificed on the already small receptor. Furthermore, patients might experience more discomfort, having an additional object to bite on or keep steady. Therefore, the aim of this study was to evaluate whether a reference step wedge is required for accurate densitometric results.

## MATERIALS & METHODS

Thirty-two dried bone samples from the premolar region of the mandible were used in this study. The samples were obtained from adult cadavers from the Department of Anatomy (Faculty of Medicine, KU Leuven, Belgium) with ethical approval.

DXA measurements were made with a fan beam Hologic QDR-4500a® (Hologic, Bedford, MA, USA; Figure 3.1), calibrated daily in accordance with the manufacturer's recommendations. The regional high-resolution mode of the small animal scan protocol (scan field 5.0 × 7.4 cm², line spacing and point resolution 0.0311 cm) was used. The specimens were positioned on a plexi support (thickness = 2.0 cm). All DXA measurements and analysis (Subregion Hi-Res V8.26 h) were performed by the same technician. The jaw bone mineral density (BMD) as measured by the DXA scan was used as the gold standard for bone density.

Intra-oral radiographs were obtained from all samples with the Prostyle Intra (Planmeca, Helsinki, Finland). Exposure parameters were 8 mA, 60 kV and 0.08 s. The Vistascan® phosphor plate technique was used for image recording (Dürr Dental, Bietigheim-Bissingen, Germany). A box was designed to standardize the projection geometry. An aluminium step wedge was placed on the X-ray receiver, next to but not in contact with the bone sample (Figure 3.2). The wedge consisted of nine steps, each increasing the height by 1.3 mm. After scanning the phosphor plates, a noise filter was applied (DBSwin software®, Dürr Dental).


**Figure 3.1:** DXA scanner with bone sample on plexi-glass

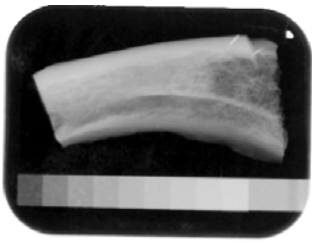


**Figure 3.2:** Radiograph resulting from experimental set-up

The radiographs were exported to a custom made software, previously described (Nackaerts et al 2006). In this software, first, the wedge must be identified by the operator on the radiographic image. Mean grey value and Al equivalent density (AED, in mm) for each step are calculated. Then, a region of interest must be selected. For the current study, the region consisted of the entire bone sample. Of this region, the mean AED was calculated. Two observers performed the analysis twice. First, the aluminium step wedge was used as a reference, and the results were expressed as AED. For the second measurement, radiographic density was the only reference, and the results were expressed as grey values. One observer repeated all measurements. To determine how few steps would be necessary to maintain accuracy, ten samples were used. The stepwedge was identified as having nine, then eight, seven, etc. steps visible on the radiograph. As such, each sample was measured nine times.

## *Statistical analysis*

Medical Statistical Software Medcalc® (Mariakerke, Belgium) was used for statistical analysis. Inter- and intra-observer variability was assessed with Passing and Bablock regression. The correlation coefficients for the DXA measurements and the measurements on intra-oral radiographs were calculated. Afterwards, the difference in predicting the areal BMD as measured by DXA was assessed for measurements with and without the aluminium reference wedge. To obtain information on the number of steps necessary to maintain accuracy, the Wilcoxon

test was used. The Bonferroni correction was performed to maintain an overall significance level of 0.05.

# RESULTS

## *Intra- and inter-observer variability*

Repeatability of the method using the aluminium step wedge as a reference for bone density measurements was previously reported (Nackaerts et al 2006) and proven to be excellent. For the method without a reference wedge, neither for intra-observer nor for inter-observer data, a significant deviation from linearity was found ($p > 0.10$) and agreement was high as visualised in Figures 3.3 and 3.4.

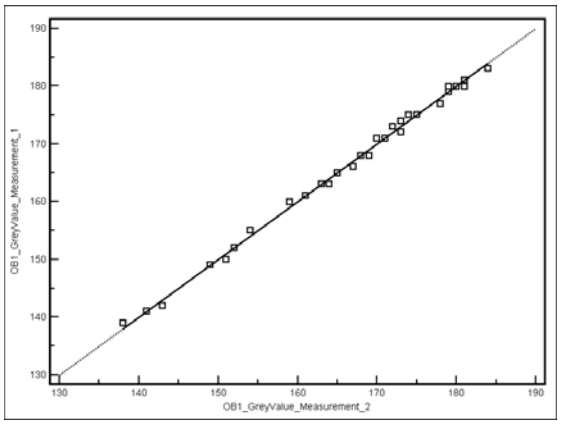



**Figure 3.3:** Passing and Bablok regression for intra-observer variability of bone density expressed as grey value

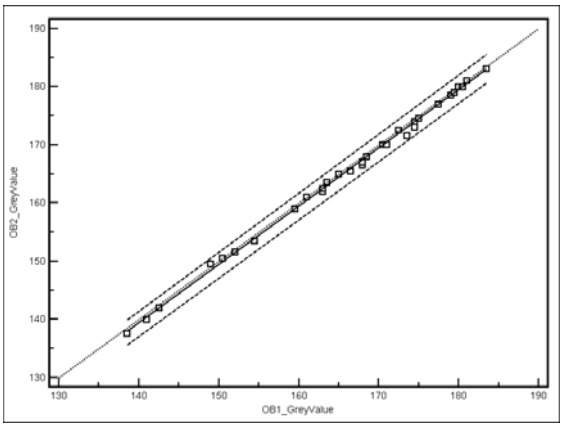



**Figure 3.4:** Passing and Bablok regression for inter-observer variability of bone density expressed as grey value

## *Densitometric measurements*

### Data description

The BMD as measured by the DXA of the mandibular bone samples ranged from 0.528 to 0.820 g/cm², with a mean of 0.661 g/cm² and standard deviation of 0.079 g/cm². A normal distribution of mandibular BMD was accepted by D'Agostino-Pearson test for normal distribution.

### Prediction of BMD

Table 3.1 shows the correlation of DXA results and density measurements on intra-oral radiographs with (AED) and without (Grey value) a reference wedge. The

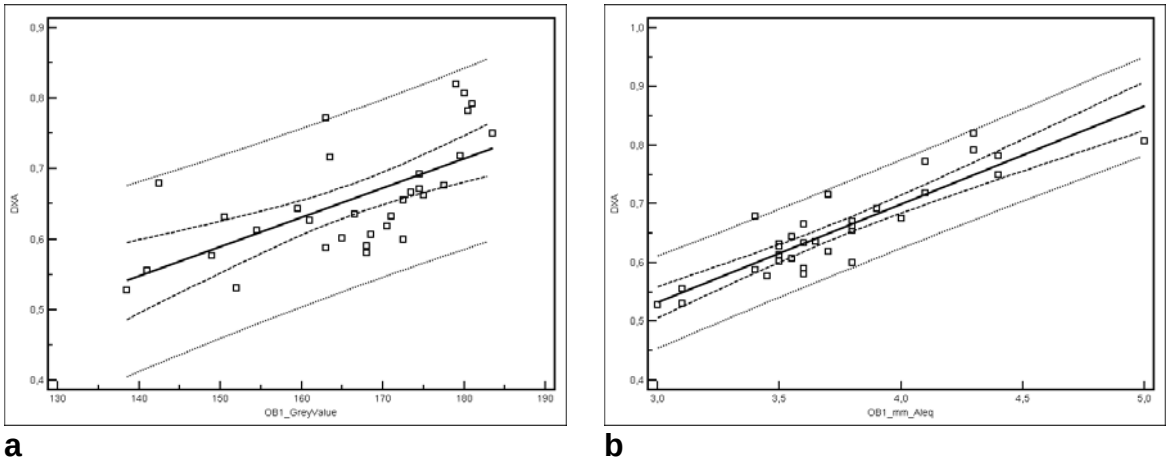
correlation between mandibular BMD and the densitometric values on intra-oral radiographs was substantially higher when the aluminium step wedge was included. Regression results confirmed that the gold standard was much better predicted when the wedge was included than without the wedge (Table 3.2, Figure 3.5).

**Table 3.1:** Pearson correlation coefficients for BMD as measured with DXA and radiographic bone density expressed as grey value and Al equivalent density (AED)

| | | **BMD (g/cm²)** |
|---|---|---|
| **Grey value** | Correlation coefficient | 0.65 |
| | Significance level p | < 0.05 |
| | n | 32 |
| **AED** | Correlation coefficient | 0.89 |
| | Significance level p | < 0.05 |
| | n | 32 |

**Table 3.2:** Linear regression analysis for bone density expressed as grey value and Al equivalent density (AED)

| | **Regression grey value** | | | | **Regression AED** | | | |
|---|---|---|---|---|---|---|---|---|
| Dependent Y | DXA | | | | DXA | | | |
| Independent X | Grey value | | | | AED | | | |
| $R^2$ | 0.43 | | | | 0.80 | | | |
| Regression equation | y = -0.0368 + 0.0042x | | | | y = 0.0322 + 0.1670x | | | |
| **Parameter** | **Coefficient** | **SEM** | **T** | **p** | **Coefficient** | **SEM** | **T** | **p** |
| Intercept | -0.04 | 0.15 | -0.25 | 0.80 | 0.03 | 0.06 | 0.56 | 0.58 |
| Slope | 0.004 | 0.00 | 4.72 | < 0.05 | 0.17 | 0.02 | 0.88 | < 0.05 |




**a** **b**

**Figure 3.5:** a) Linear regression line for grey values predicting BMD; b) Linear regression line for AED values predicting BMD. The dotted line represents the 95% CI, the striped line represents the 95% prediction interval

### Number of steps

The Wilcoxon test revealed no significant difference between the density measurements using nine to three steps. With the use of two steps, it was impossible to calculate the AED. The correlation between a 3-step aluminium wedge (i.e. first three steps) and the mandibular BMD was 0.84 ($p < 0.05$).

## DISCUSSION

A tool for jaw bone densitometry on intra-oral radiographs, using an aluminium step wedge as a reference, was previously proven to have good intra- and inter-observer repeatability (Nackaerts et al 2006). Using grey values for bone density measurements within the same custom software has now also proven to be a reproducible method. Although a correlation does exist between the mere grey value and the DXA results, it is far less strong than the correlation between AED value and the DXA results. Moreover, when performing linear regression, only 43% of BMD variation is explained by grey values, in contrast to the 80% explained by AED values. Therefore, we conclude that jaw bone density assessment based on grey values, even with a brightness correction, is not an acceptable measure.

DXA was chosen as a gold standard, because it was found to be a good reference in several studies concerning jaw BMD (Corten et al 2004, Horner & Devlin 1998). An aluminium wedge was used because of its similar absorption and scatter properties of bone (Trouerbach 1984) and previous applications in similar research (Du Tré et al 2006). Other suggested materials are hydroxyapatite and barium sulfate (Yang et al 2002). Nickel was also used as a reference material in oral research context (Horner & Devlin 1998). The use of materials with a higher atomic number, such as nickel, might avoid the inclusion of the rather thick aluminium wedge when taking intra-oral radiographs. Because space is limited on intra-oral films, exploratory research should first ensure that the scatter does not deform the bone properties. As three steps were sufficient to maintain an accurate bone density measurement, not only thickness, but also length of the wedge could be diminished to a large extent.

To implement the results of this study into clinical practice, it could be considered to build in a reference material in all intra-oral films. This should be a

small object, showing a range of densities, possibly including various materials with different absorption properties. Population-based normal density values could then be obtained and used as a starting point for bone mass evaluation, e.g. in preoperative implant planning, bone gain or bone loss because of local and/or systemic diseases or in predicting skeletal bone density. The inclusion of a reference material could also be valuable to the dentist as an instrument for quality control.

In conclusion, densitometric analysis showed good reproducibility, for the analysis with and without aluminium wedge correction. However, the assessment of bone density was far more accurate with the tool including an aluminium reference wedge. Three steps appear to be sufficient for bone density evaluation. Further research needs to be performed to develop the most clinically applicable tool for densitometry on intra-oral radiographs.

## ACKNOWLEDGEMENTS

This work was supported by a research and technological development project grant from the European Commission FP5 ‘Quality of Life and Management of Living Resources’ (QLK6-2002-02243 OSTEODENT). Grateful acknowledgement is given to Herman Borghs from the Department of Bone Metabolic Disease.

# Chapter 4

## Accuracy and precision of a densitometric tool for jaw bone

## INTRODUCTION

Bone mineral density (BMD) measurements are crucial in the early diagnosis of osteoporosis. A timely recognition might lead to a considerable decrease in osteoporosis-related community costs. It would enable prevention of fragility fractures, which are known to increase the risk for subsequent fractures (Haentjens et al 2003). A mass screening of post-menopausal women would therefore be ideal. For this purpose, a number of clinical prediction rules (CPR) to identify women with low BMD exist. Recent studies showed these CPRs to have too low specificity (Mauck et al 2005). Dual-energy X-ray absorptiometry (DXA) and other BMD measurement tools have been modified for peripheral measurements, making these more appropriate for screening purposes in the general practitioners' office (Bonnick 2004). This kind of screening, however, still depends upon interest in bone status both from the practitioner's and patient's point of view.

Researchers have shown an interest in oral radiographs as valid screening instruments to identify women or men with low BMD (von Wowern 2001, White 2002). The choice for oral radiographs can easily be justified, since these are readily available in most patients' dental files. Furthermore, correlations between oral radiographic indices and skeletal bone status have been documented in several publications (Horner et al 1996, Jacobs et al 1996, Klemetti et al 1997, Southard et al 2000, Jonasson et al 2001, Devlin & Horner 2002, Horner et al 2002, Jonasson & Kiliaridis 2005, White et al 2005). Nevertheless, large-scale studies are required to obtain more irrefutable results and to develop a clinically applicable tool for daily dental use (Mohajery & Brooks 1992, Drozdzowska et al 2002). With such a tool, the dentist could play a role as primary care clinician for referral of post-menopausal women at high risk for osteoporosis and as such save time and money for the community. The latter becomes more important as the population is ageing, which increases the prevalence of the disease.

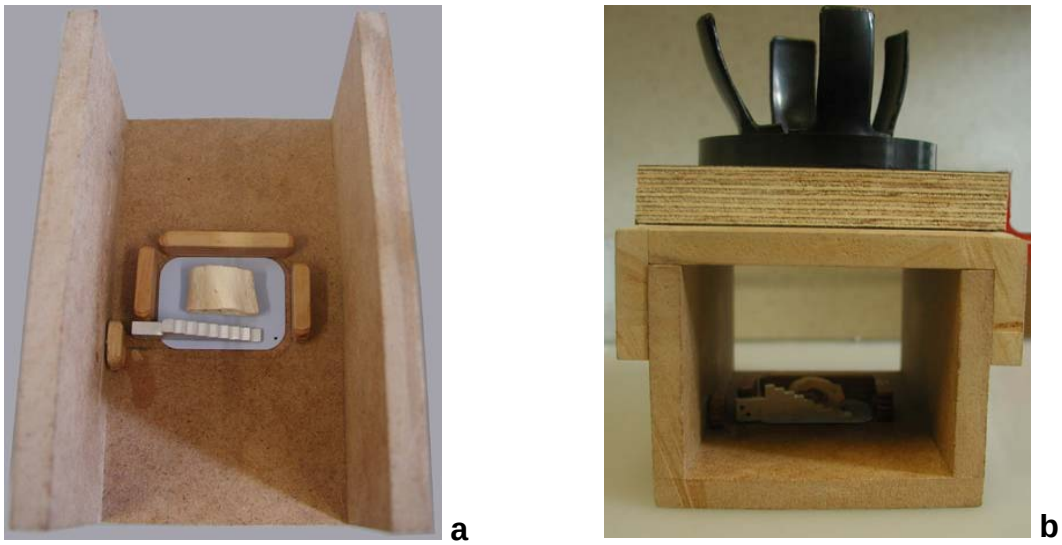
The aim of the present study was to develop a method for densitometric measurements using digital intra-oral radiographs. The densitometric tool was tested for its potential use in upcoming clinical studies: accuracy and precision were determined by means of *in vitro* validation. Finally, minimal detection threshold of the tool was determined, applying osteoporosis simulation by

decalcification. This experimental set-up was, to our knowledge, only used by Southard and Southard (1994) before.

## MATERIALS & METHODS

### *Materials*

The material consisted of 47 human bone samples of the premolar region. The size of the samples varied, but had an average of 1.5 cm³. Samples were obtained from adult cadavers from the Department of Anatomy (Faculty of Medicine, KULeuven) with ethical approval. All samples were defatted (Compound Cleaner®; Castrol, Wayne, NJ) and oven dried.



**a** **b**

**Figure 4.1:** Wooden box for standardized radiography. (a) Aluminium wedge is included in all radiographs; (b) Collimator fits cover of box

To standardize the projection geometry, a wooden box was designed including a film holder and an opening to fit a rectangular collimator. To enable brightness and contrast standardization as well as to allow comparison of the densitometric values, an aluminium step wedge was placed on the X-ray receiver (Figure 4.1). The bone sample was placed on the receiver, though never in direct contact with the wedge to avoid scattering. Digital radiography was performed on all samples with the VistaScan® phosphor plate technique (Dürr Dental, Bietigheim-Bissingen, Germany) using a Prostyle Intra® radiation tube (Planmeca, Helsinki, Finland) at 60 kV, 8 mA and 0.08 s. After scanning, the noise filter from the DBSwin software®

(Dürr Dental, Bietigheim-Bissingen, Germany) was used. Image analysis was performed using custom-made densitometric software. The software considered the reference stepwedge included in the image. Output consisted of the medium pixel value of the aluminium steps and Al equivalent density (AED, in mm) of the region of interest (ROI) selected. In the current study, ROI consisted of the entire bone specimen.

### *Methods*

For validation of the densitometric tool, precision, accuracy and minimal detection threshold were calculated.

## Precision & accuracy

For intraobserver reliability, two observers measured the bone sample density three times for all 47 specimens, each time with a 2-week interval. For interobserver reliability, the results of analysis of two independent observers were compared.

To obtain two-dimensional (2D) reference density values, DXA scans were made of the bone samples, using a fan beam system Hologic QDR 4500a® (Hologic Inc., Bedford, MA).

To obtain three-dimensional (3D) reference density values, specimens were weighed dry and submerged with a Sartorius 2004MP® balance (Sartorius, Vilvoorde, Belgium). The volume was derived by Archimedes' principle. Volumetric density could thus be calculated: ρ = dry mass (g)/volume (mm³).

## Minimal detection threshold

To determine the minimal detection threshold of the method, four bone samples were progressively decalcified with a HCl solution (Decal®; Serva, Heidelberg, Germany) to simulate osteoporosis. The Ca level at each decalcification interval was determined, as well as Ca content after full decalcification. As such, the percentage of Ca that was lost at each interval could be calculated. At each interval, radiographs were taken and analysed with the software. The minimal change detectable on the radiographs was determined and associated with the amount of Ca loss this change required. For this part of the

study, F-speed intra-oral films (Kodak, Rochester, NY) were used. The films were developed with an automatic film processor (XR 24 Nova®; Dürr Dental, Bietigheim-Bissingen, Germany) using fresh chemicals (Dürr Automat XR®) including automatic regeneration. These were scanned afterwards (Snapscan®; Agfa, Mortsel, Belgium) in transparency mode, at a resolution of 600 dpi. Using the densitometric software, the percentage of visible decalcification on the radiographs was calculated to find the minimal detection threshold for density changes. The percentage of actual decalcification was obtained using the Ca content after full decalcification (total Ca content) and calculation of the percentage of Ca loss after each decalcification step.

### *Statistical analysis*

For statistical analysis, Statistica® (Statistica version 6, StatSoft Inc., Tulsa, OK-USA) was used. Precision of the method, using repeated measurements, was calculated with the coefficient of variation (CV = 100*(standard deviation)/(mean value of set)). For interobserver variability, Passing and Bablok regression was performed on the data set. The correlation coefficient for DXA and AED results was calculated. Further analysis of these data was done using linear regression.

## RESULTS

### Precision

CV calculations were below 3% for each testing group, pointing to a good precision of the method (Table 4.1). The results for interobserver variability are shown in Figure 4.2 and Table 4.2. The regression equation (y = A + Bx) being y = 0 + 1x, with the 95% confidence interval for A = 0 containing 0 and the 95% confidence interval for B = 1 containing 1, proves the agreement between observers.

**Table 4.1:** Intra-observer variability of the densitometric tool

| Test data | $CV_{rms}$ % |
|---|---|
| Observer 1 | 0.84 |
| Observer 2 | 2.54 |
| Observer 1 | 0.93* |
| Observer 2 | 0.71* |

* Repositioning of the samples

**Table 4.2:** Passing and Bablok regression for interobserver agreement

| | |
|---|---|
| **Regression equation** | y = 0 + 1x |
| **Intercept A** | 0 |
| **95% confidence interval (CI)** | -0.11 to 0 |
| **Slope B** | 1 |
| **95% CI** | 1 to 1.03 |
| **Cusum test for linearity** | No significant deviation from linearity (p > 0.10) |

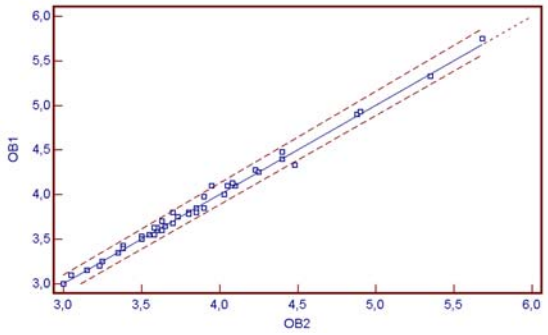



**Figure 4.2:** Passing and Bablok regression plot, showing close interobserver agreement

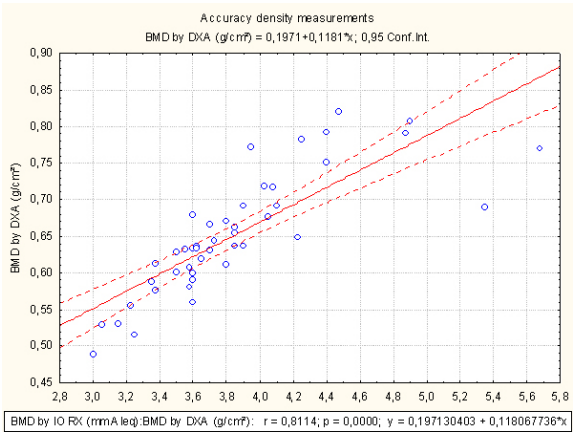



**Figure 4.3:** Scatterplot showing linear relation between AED values and bone mineral density (BMD) data.

## Accuracy

The correlation coefficient between AED values and the areal BMD results of the DXA scan was 0.81 ($p < 0.05$). Linear regression was applied on the data and the following model was constructed to predict aluminium values with BMD: BMD by DXA = 0.19 + 0.12 x (BMD by intra-oral radiography) (Figure 4.3). This formula then allowed an estimate of the BMD value of the jaw bone based on AED as determined by intra-oral digital radiology and densitometry.

For the volumetric density data, the correlation coefficient with AED was 0.5, though not significant.

## Minimal detection threshold

The custom-made software was able to detect an actual change in bone mineralization of 6.6%. The change in AED, however, was not parallel to the change in mineralization, as is visible in Figure 4.4.

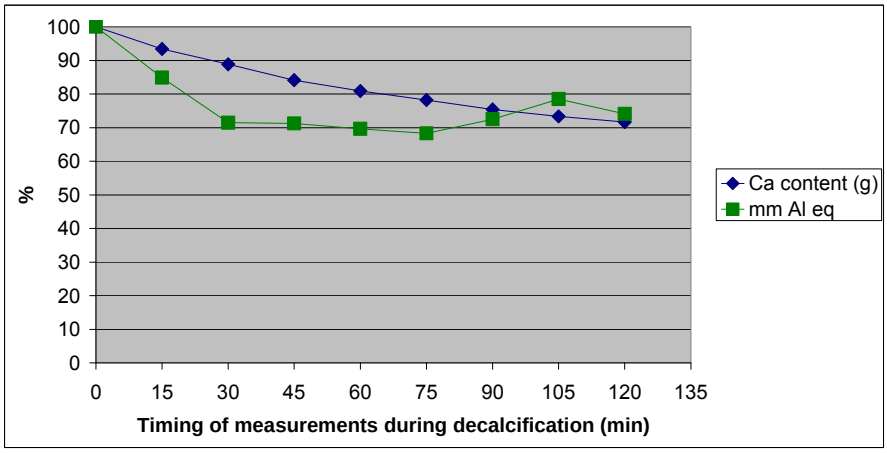



**Figure 4.4:** Inter-related decrease of percentage radiological density (AED) and percentage Ca content during progressive decalcification

# DISCUSSION

A number of studies focusing on bone density and monitoring bone remodelling have used a step wedge for reference (Jacobs et al 1996, Trouerbach et al 1984, Dornier et al 2004). These methods were usually successful in evaluating small bone density changes. The novelty of the tool currently under validation, lies in its potential clinical applicability, possibly to be extended from the research-friendly context of the university hospitals towards general practice. Until today, dentists are mainly inspecting radiographic bone density changes on a pure subjective visual basis. On the other hand, image processing based on more objective parameters (reference wedge) remains a pure research tool, while the presence of a reference material could make the clinicians' judgement on patient status and the evolution of this status more scientifically founded. The use of intra-oral radiographs, being readily available for most general dentists, enlarges the potential field of implementation. With ongoing technological progress, software updates have become indispensable to enable large scale studies relating skeletal

and oral bone density in a way that it reliefs the user of numerous manual manipulation of the images.

Our custom-made tool might serve this purpose. The precision and interobserver reliability of the densitometric tool are most satisfactory. The highly significant correlation between AED data and DXA results demonstrates the necessary accuracy of this newly developed densitometric tool. The low correlation between AED data and volumetric data might be explained by the method used to obtain the sample volume; submersion in water of bone is possibly a too inaccurate method, certainly when working with minute data as we did in the present study.

DXA measurements were used as the gold standard for bone density, since it is considered the gold standard in bone density research worldwide. The precision of DXA measurements is very high, ranging from 0.9% to 2.3%, which is a higher precision than the one that was obtained in this study. The accuracy of DXA to measure actual bone density is 3–6% (Bonnick 2004). The present method for bone densitometric analysis offers potentials for evaluation of bone density and minute bone density changes in the jaw bone. Although performed on a small sample, the detection threshold of bone loss of 6.6% seems to be promising. Annual perimenopausal bone loss is reported to be a 2-9% BMD decrease (Elders 1988, Pouilles et al 1993, Bainbridge et al 2002). This means the change that can be detected with the current tool is clinically relevant. Ongoing research on a large-sample clinical data set may prove its applicability in dental practice. This approach may also allow construction of a normative reference database for jaw bone of the focused female population.

In a clinical study, especially with a longitudinal design, additional methodological challenges arise. First of all, soft tissue is included in the radiograph, and its properties might change over time. Second, geometric standardization is not self-evident when taking radiographs in patients. Working with impression material, such as stent, could offer a solution to this problem, but it is not flawless, since it takes no account of the possibility of teeth that are lost or that have changed position. In addition, the equipment and the radiographers can vary over time. Finally, patient drop-out should be considered in a longitudinal study design.

Previous studies on the detection of bone loss on conventional 2D radiographs show that 30–53% of the bone can be removed before a difference could be noted (Dryer 1993). Most of this bone density research is conducted within a framework of periodontal bone lesion and healing follow-up (van der Stelt et al 1991). Furthermore, pre-operative planning of implant placement and the evaluation of implant osseointegration depend on valuable radiographic bone mass evaluation. To study bone lesion and healing follow-up, localized defects are drilled in bone samples, requiring bone volume rather than bone density evaluation (van der Stelt et al 1991). As far as we could retrieve, only Southard and Southard (1994) performed decalcification studies comparable with the present study. The latter group found a 5.3% threshold. For improved osteoporosis simulation, the decalcification of the cortex should be prevented in the early decalcification stage, since trabecular bone is firstly affected by the bone disease because of faster metabolism.

Other software is available for the evaluation of intra-oral radiographs (Lehmann et al 2002). Digital radiographic systems come with software that enables image enhancement and manipulation. However, as there is no reference material included, true standardization of grey values is compromised. The inclusion of the aluminium wedge in a beam aiming device may offer great potential for future large-scale studies on BMD.

In conclusion, the presently introduced densitometric tool may offer some potential for bone density evaluation to monitor bone healing or detect early signs of osteoporosis. Its clinical applicability will be further examined in an ongoing study on a large female patient sample.

## ACKNOWLEDGEMENTS

This study forms a part of the OSTEODENT project, supported by the European Commission through the 5th Framework Program ‘Quality of Life and Management of Living Resources’ (QLK6-CT-2002-02243).

# Chapter 5

## Osteoporosis detection using intra-oral densitometry

## INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone density and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility (Consensus development conference 1991). More than 75 000 000 people in Europe, Japan and the USA are affected (WHO 1997). Not only does bone fragility imply a higher risk of fracture due to minimal trauma, but patients may also become bed-bound with secondary complications that may be life threatening in the elderly (Silverman 2005). Furthermore, osteoporosis causes back pain and loss of height, due to vertebral fractures (Watts 2001). As life expectancy increases, with the elderly representing a greater proportion of society, the prevalence of osteoporosis shows an upward trend. As a consequence fragility fractures, subsequent morbidity, medical costs and mortality risks also increase. This conclusion urges greater efforts in preventive measures and early detection (Melton et al 2005). The diagnosis of osteoporosis is based on bone mineral density (BMD) as measured by DXA. The World Health Organization set the threshold for diagnosis at a BMD of 2.5 standard deviations or more below the young normal mean. Although DXA measurements are necessary in order to diagnose a patient with osteoporosis, these are considered less suitable for large-scale screening because of the cost and region-dependent availability. In any case, to comply with the need for early detection, researchers have been searching for tools that allow efficient and low-cost screening for osteoporosis (White 2002, Richy et al 2004).

Among the proposed screening tools are oral radiographs. Dental practitioners regularly use panoramic and intra-oral radiographs for diagnostic purposes. As such they are readily available for the vast majority of patients, which makes these radiographs ideal for large-scale osteoporosis screening. A multiplicity of studies has been conducted about the application of existing oral radiographs to help diagnose patients with osteoporosis (Law et al 1996, Horner et al 2002, Nakamoto et al 2003, Faber et al 2004, Dervis 2005, Lee & White 2005, White et al 2005, Devlin et al 2007a, Devlin et al 2007c, Geraets et al 2007, Horner et al 2007, Karayianni et al 2007). Results mostly show that indices based on oral radiographs can be used to detect high-risk patients who should benefit from bone density measurements. In other words, the dentist might be able to play an

important role as primary care clinician, responsible for the referral of women with a high risk of osteoporosis.

Some controversy remains about the role of oral radiographs in the detection of osteoporosis. The development of a reliable diagnostic tool for daily clinical use requires extensive studies to prove its consistency and diagnostic performance. The feasibility of transfer to the clinical field needs additional verification.

The aim of the present study was to evaluate clinically a tool for jaw bone densitometry using intra-oral radiographs. The intra-oral radiographs were provided with an aluminium step wedge as a density calibration, necessary to rule out density differences due to imaging parameters (Nackaerts et al 2007). This tool has already been developed and validated *ex vivo* (Nackaerts et al 2006). The conclusion of the *ex vivo* studies was that the method for bone densitometric analysis offered potential for clinical evaluation of bone density and the detection of minute bone density changes in the jaw bone. In the reported research project, the accuracy of jaw bone density measurements in predicting osteoporosis was analysed in a large clinical study.

## MATERIALS & METHODS

Five European centres joined in OSTEODENT, a research project of which the goal was to determine the best radiographic, or combination of clinical and radiographic, method for identifying those individuals most at risk of osteoporosis. Over a 2–year period, 671 women in the age range of 45–70 years were recruited to the centres located in Manchester (UK), Malmö (Sweden), Athens (Greece) and Leuven (Belgium). The four recruiting centres received ethical approval for the study and informed consent was obtained from all subjects. The participating women were patients attending dental clinics, respondents to leaflets inviting volunteers to enter the study, or were informed by other attendees. In response to an insufficient proportion of subjects with osteoporosis recruited during the first year, an additional positive recruitment strategy was introduced. Ethical approval was obtained to recruit patients at bone densitometry departments who had been previously diagnosed with osteoporosis. No patients were included with possible secondary osteoporosis, primary hyperparathyroidism, poorly controlled thyrotoxicosis, malabsorption, liver disease or alcoholism.

DXA scans of the left hip and lumbar spine (L1 to L4) were carried out on each subject to establish the reference standard for the study. Scans were performed by experienced technicians on Hologic QDR 4500, Hologic Discovery (Hologic Inc., Bedford, MA) and GE Lunar Prodigy (GE Lunar Corporation, Madison, WI) at the four centres throughout Europe. Shewarts rules were used to monitor quality assurance throughout the study period (Orwoll & Oviatt 1991).

The European Spine Phantom (ESP, Figure 5.1) was used to standardize measurements between different manufacturers using the method described by Pearson and colleagues (Pearson et al 1995). T and Z scores were calculated using Hologic reference data for the lumbar spine and NHANES (National Health and Nutrition Examination Survey) reference data for the hip (femoral neck and total hip) (Looker et al 1998). Standardization of BMD measurements was performed by one experienced scientist and results from all four centres were reviewed and confirmed by one clinical radiologist with expertise in this field. Subjects were diagnosed as osteoporotic according to the World Health Organization (WHO) criteria (WHO 1994). By using this definition, subjects with a T score value 2.5 standard deviations (SDs) or more below the mean bone mineral density (BMD) value of the young gender-matched reference population at any one of total hip, femoral neck or lumbar spine were considered as osteoporotic and all others as healthy.

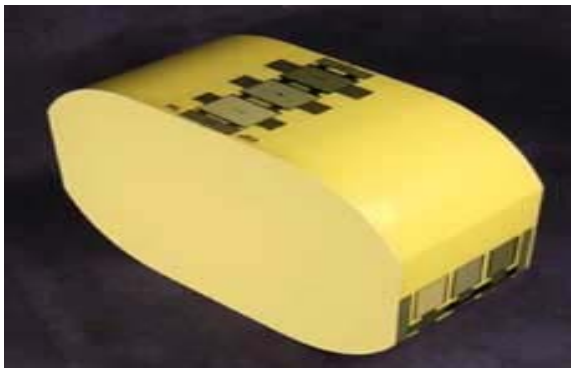


**Figure 5.1:** European Spine Phantom

**Figure 5.2:** Film-holding instrument with Al wedge

Intra-oral radiographs were made in all subjects. More specifically, one periapical radiograph of the right premolar region in the upper and one in the lower jaw were taken. In three centres, Planmeca Prostyle Intra® devices were used

(Planmeca Oy, Helsinki, Finland, 60–63 kV, 8 mA), and in one centre a Siemens Heliodent MD (Sirona, Bensheim, Germany, 60 kV, 8 mA). Rinn XCP® (Dentsply, York, PA) film-holding instruments were adapted to contain an aluminium step wedge to standardize radiographs throughout all participating centres and to allow densitometric analysis (Figure 5.2). The conventional films were scanned at a resolution of 118 pixels/cm (300 pixels/inch).

The digitized radiographs were analysed with custom-made software, previously described and thoroughly tested *ex vivo* (Nackaerts et al 2006). The region of interest (ROI) was drawn interactively on computer, covering the interdental bone of the right premolars of the upper and lower jaw. The ROI included trabecular bone only and was drawn to include as much trabecular bone as possible without involving the lamina dura, the periodontal ligament or the root. In cases where premolar teeth were adjacent to each other, leaving no or hardly any interseptal trabecular bone, the ROI of that particular location was not analysed. When no teeth were present, the ROI was drawn in the same area, making sure that no extraction sockets were involved. For radiographs in which bone pathology was visible, extra attention was paid to avoid including affected bone. If selecting a ROI was not possible due to too large a bone lesion, the ROI was not analysed. The software calculated the mean pixel value of the aluminium steps and the Al equivalent density (AED, in mm) of the selected ROI. For interobserver variability, a second observer measured 15% of the images. For intraobserver reliability, observer one measured the bone density twice on 60 randomly chosen radiographs.

## *Statistical analysis*

Medical Statistical Software Medcalc® (Mariakerke, Belgium) was used for statistical analysis.

Intra- and inter-rater repeatability were assessed with the concordance correlation coefficient.

Receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic performance of jaw bone density measurements for diagnosis of osteoporosis at any measured site (lumbar spine, femoral neck or total hip). The value for the area under the ROC curve ($A_z$) can be interpreted as follows: an area

of e.g. 0.84 means that a randomly selected individual from the positive group has a test value larger than that for a randomly chosen individual from the negative group 84% of the time (Zweig & Campbell 1993). When the measurements have no diagnostic value at all, the area would be 0.5; an area of 1 indicates a perfect ability to classify subjects in the correct group. From 0.7 to 0.8, the test is considered to have fair accuracy; and above 0.8, it is considered to be a good test. The cut-off value is the value above which subjects are classified in the negative group (healthy), and below which subjects are classified in the positive group (osteoporosis). Depending on the choice of the cut-off value, sensitivity (true positive rate) and specificity (true negative rate) will vary.

## RESULTS

Out of the 671 recruited post-menopausal women, 8 women did not match the age criteria and in 2 women, data on total hip BMD were missing. Thus, 661 women were considered for analysis. Their mean age was 54.9 years (SD = 6.08). A total of 141 (21.3%) subjects were classified as having osteoporosis involving at least one site (total hip, femoral neck, or lumbar spine). A summary of the density results for subjects with and without osteoporosis at one or more of the measured skeletal sites is shown in Table 5.1 and Figure 5.1. The mean AED value was significantly lower for women with osteoporosis than for healthy women ($p < 0.0001$).

**Table 5.1:** Subjects' jaw bone density and dual energy X-ray absorptiometry results. Data are grouped by osteoporosis status. Differences are indicated

| | Healthy | | Osteoporosis | | |
|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Diff*** |
| Upper jaw density (AED, mm) | 5.66 | 1.32 | 4.67 | 1.25 | 0.99 |
| Lower jaw density (AED, mm) | 5.66 | 1.45 | 4.61 | 1.54 | 1.05 |
| BMD Femoral Neck (g/cm²) | 0.79 | 0.12 | 0.58 | 0.08 | 0.21 |
| BMD Total Hip (g/cm²) | 0.91 | 0.13 | 0.69 | 0.09 | 0.22 |
| BMD Lumbar Spine (g/cm²) | 1.02 | 0.15 | 0.72 | 0.08 | 0.30 |

*All significant ($p < 0.0001$)

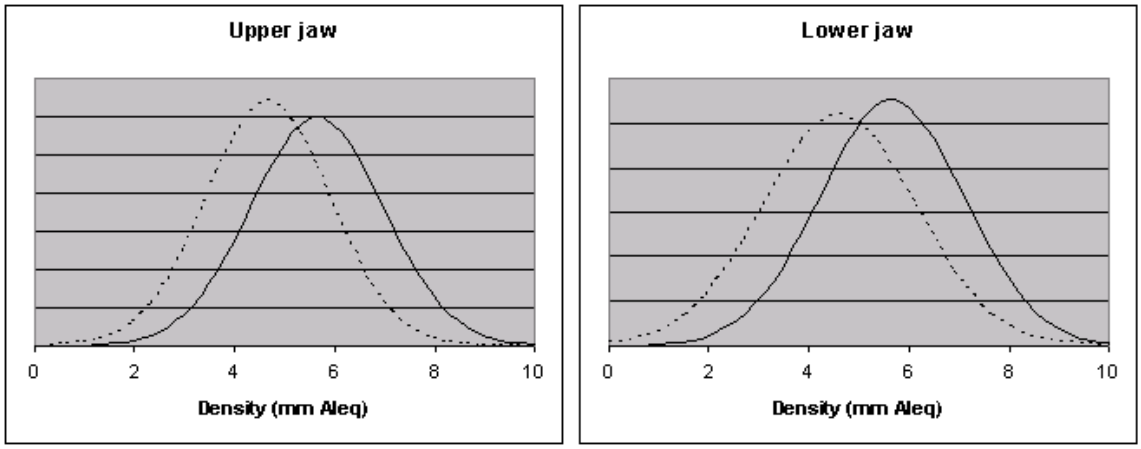



**Figure 5.3:** Bell curves for upper and lower jaw bone density measurements, separately for subjects with (dashed curve) and without (full curve) osteoporosis

For the upper jaw, 602 radiographs could be analysed, meaning that 9% of values were missing. 8% of the dropouts were due to insufficient trabecular bone available for measurements or misplacement of the aluminium wedge on the image. 1% were rejected because of image quality (over- or underexposure, artefacts). The result of the ROC curve, using upper jaw radiographs for osteoporosis diagnosis, was an area of 0.705 (95% confidence interval (CI) 0.667–0.742; Figure 5.2). For a cut-off value of 4.3 mm AED, sensitivity was 38.7% and specificity 83.5.

For the lower jaw, 574 radiographs could be analysed. This meant that 13% of the values were missing, 11% of which were due to image quality characteristics, as mentioned before, and the other 2% due to extreme results. The result of the ROC curve, using lower jaw radiographs for osteoporosis diagnosis, was an area of 0.689 (95% CI 0.650–0.727; Figure 5.3). For a cut-off value of 4.3 mm AED, sensitivity was 33.9% and specificity 85.3%.

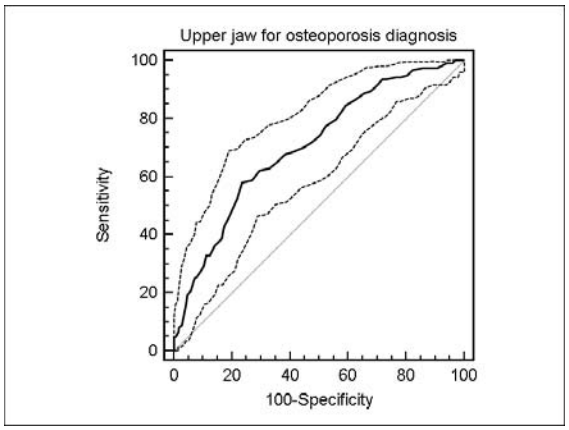



**Figure 5.4:** ROC curve for upper jaw bone density. The full line represents the ROC curve for upper jaw bone density measurement as a predictor for osteoporosis at any measured site ($A_z$ = 0.705). The dashed lines represent the 95% confidence interval

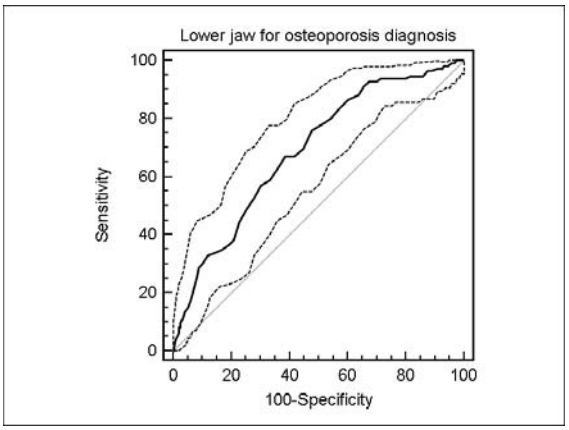



**Figure 5.5:** ROC curve for lower jaw bone density. The full line represents the ROC curve for lower jaw bone density measurement as a predictor for osteoporosis at any measured site ($A_z$ = 0.689). The dashed lines represent the 95% confidence interval

The concordance correlation coefficient for intrarater agreement was 0.98 for the upper and 0.95 for the lower jaw. For interrater agreement, the concordance correlation coefficient was 0.93 for upper jaw measurements and 0.87 for lower jaw measurements.

## DISCUSSION

For detecting osteoporosis at the total hip, femoral neck or lumbar spine using jaw bone density, the area under the ROC curve ($A_z$) was 0.7. This area indicates a fair diagnostic accuracy of the density measurements. From the overlap as seen on the bell curves, it is clear that jaw bone density is not an excellent distinctive characteristic for osteoporosis. Many reports exist about the performance of oral radiographs for identifying osteoporosis in patients. Mostly, these studies involve indices deducted from panoramic radiographs. Sensitivity and specificity are difficult to compare because of altering cut-off values and test variables, but $A_z$ are mostly between 0.7 and 0.8 (Devlin & Horner 2002; Lee et al 2005; White et al 2005; Devlin et al 2007a; Devlin et al 2007c; Geraets et al 2007; Horner et al 2007; Karayianni et al 2007). Reports on jaw bone density derived from intra-oral radiographs to detect osteoporosis are less widespread, but mostly report mediocre diagnostic validity (Mohajery & Brooks 1992; Jacobs et al 1996; Southard et al 2000; Jonasson et al 2001; Lee & White 2005). Higher predictive

values were found when analysing the trabecular pattern of intra-oral radiographs (White et al 2005; Geraets et al 2007). A combination of structural (Geraets & van der Stelt 1991; Geraets et al 1993; Geraets et al 1998) and densitometric analysis might yield more convincing results for predicting osteoporosis. This should be further investigated.

The current cut-off level of 4.3 mm AED was chosen so as to obtain high specificity scores. Most clinical risk indices that are in use have high sensitivity though poor specificity (Cadarette et al 2000; Geusens et al 2002; Sedrine et al 2002; Mauck et al 2005). This means that quite a number of healthy individuals are referred for bone density measurement. These indices, such as OSIRIS (Osteoporosis Index of Risk) and ORAI (Osteoporosis Risk Assessment Instrument), are used specifically to determine whether such referral is suitable. Oral radiographs on the other hand, are acquired for other diagnostic purposes. Patients do not expect to be alerted about possible low systemic bone density by their dentist. It is therefore important not to worry healthy patients, which can be achieved with a highly specific test. Another reason to opt for high specificity is the expense and the region-dependent availability of DXA scanners, the rationale for the current study.

Maxillary and mandibular densitometric measurements showed no significant difference in predicting osteoporosis. It seems that there is no preferred jaw to predict osteoporosis at the lumbar spine, femoral neck or total hip. This appears to be controversial. Indeed, Southard et al (2000) found a relationship between maxillary density and systemic bone density, while this relationship was missing for mandibular density. Most researchers only involve mandibular measurements in the analysis, since the area of the mandible posterior to the mental foramen is considered the standard measurement site for jaw bone analysis. The reason is that it has the smallest inter- and intraindividual variations in anatomical size, shape, bone structure and function (von Wowern 2001).

Including an aluminium wedge on an intra-oral radiograph was a challenge: although the bite blocks were adapted to the wedge, many radiographs could not be measured because teeth were overlapping the wedge, or the wedge was partially or fully out of the projection field. Still, with a missing value rate of 9% (upper jaw) and 13% (lower jaw), we were not too far from the targets for

radiographic quality from the 2001 Guidance notes for dental practitioners on the safe use of X-ray equipment, stating that a rejection rate of radiographs should not exceed 10%. If a standard tool is to be developed for jaw bone density measurements on intra-oral radiographs, an exploratory study would be advisable to find another way of including a reference material. This could be a material with a higher atomic number, such as nickel or copper (Horner & Devlin 1998; Lee & White 2005). Another option is to adapt the image receptor with an in-built reference. In practice, it might be more practicable using a solid-state digital receptor rather than with film.

In conclusion, jaw bone density as measured with the current tool yielded fair diagnostic accuracy for osteoporosis in the hip or spine. By increasing the sensitivity and specificity it should be possible to obtain a more reliable pre-screening tool. A non-exhaustive list of measures to reach that includes the use of structural characteristics of jaw bone and possibly clinical data, the use of an image receptor with a built-in calibration tool and software that is automated to a high extent so as to minimize user interference. Ultimately, replicability analyses should confirm the validity of the method.

## ACKNOWLEDGEMENTS

This work was supported by a research and technological development project grant from the European Commission FP5 ‘Quality of Life and Management of Living Resources’ (QLK6-2002-02243 OSTEODENT).

# Chapter 6

## Is self-reported alcohol consumption associated with osteoporotic mandibular bone loss in women?

## INTRODUCTION

Excessive alcohol (Diamond et al 1989) consumption has long been recognized as a risk factor for osteoporosis. Chronic alcohol consumption has an adverse effect on the function and differentiation of osteoblasts, favouring the production of adipocytes instead, whereas moderate alcohol consumption has a beneficial effect on bone mineral density (BMD) by increasing serum estrogen (Tannirandorn & Epstein 2000). Rapuri et al (2000) showed that parathyroid hormone secretion may be reduced in those with moderate alcohol consumption, resulting in decreased bone resorption and increased BMD. Similarly, moderate alcohol consumption was found, in a prospective study, to have a beneficial effect on BMD (Holbrook & Barrett-Connor 1993). Moderate alcohol consumption may reduce the rise in bone resorption that is found in postmenopausal osteoporosis. Co-factors, such as smoking, liver disease, poor nutrition, and other lifestyle factors may be important as many binge drinkers also smoke heavily. Smoking is accepted as a risk factor for tooth loss (Gomes-Filho et al 2007) and implant loss, but also has systemic effects on skeletal metabolism. Smoking can cause a reduction in the serum levels of 25-hydroxy vitamin D and, consequently, reduce the BMD (Brot et al 1999).

We have previously shown that the thickness of the mandibular cortex on dental panoramic radiographs can be used as an indicator of osteoporosis, with manual measurements having moderate accuracy in predicting osteoporosis. Karayianni et al (2007) used the area under the receiver operating characteristic curve ($A_z$), where the greater the area, the more useful the diagnostic test in identifying patients with osteoporosis. They found values of $A_z$ = 0.71–0.78, which were significantly better than chance at predicting disease. These measurements can be automated using computer software to detect the image of the lower mandibular cortex and measure its width (Devlin et al 2007a; $A_z$ = 0.76). When information about the cortical thickness was combined with various clinical risk factors, the resulting algorithm was a powerful predictor tool for osteoporosis (Devlin et al 2008; $A_z$ = 0.90). However, previous work has shown that mandibular BMD is influenced by the age of the patient (Devlin et al 2007b) and therefore this must be taken into account as a confounding variable in any analysis.

The aim of this study was to determine, in a cross-sectional study of a large European female population (45–70 yr of age), whether alcohol consumption would predict mandibular bone quantity and quality.

## MATERIALS & METHODS

In total, 672 women were recruited from centres located in Manchester (UK), Malmö (Sweden), Athens (Greece), and Leuven (Belgium) with ethical approval from the respective ethical committees. The women were patients attending the dental clinic, respondents to leaflets inviting volunteers, or were informed by other participants of the study. Full details of the recruitment procedures have been reported previously (Karayianni et al 2007). Informed consent was given by all participating women. Nine patients were eliminated from the analysis because they were either outside the age range of 45–70 yr or their age was not recorded, leaving a sample of 663 women.

The patients were asked to complete a questionnaire on various medical and lifestyle factors (including alcohol consumption) and family history. The questions on alcohol consumption were phrased as follows:

- Do you drink alcohol? Never/occasionally/regularly
- If regularly, approximately how many units per week? 1–14 units/more than 14 units

One unit of alcohol represents 10 ml by volume of pure alcohol or about half a pint of ordinary strength beer (3–4% alcohol by volume). After the patient had completed the questionnaire, the researcher went over the questionnaire with the patient to ensure that it had been completed and to answer any questions.

This method of self-selected sampling attracted individuals who tended to have a healthy metabolic bone status and a healthy lifestyle. To overcome this selection bias, one centre increased the percentage of osteoporotic individuals in the sample by recruiting those individuals who had been previously diagnosed with osteoporosis. This resulted in a sample with approximately the same percentage of osteoporotic individuals as reported in large female populations of a similar age (WHO, 1994).

Panoramic radiographs of 645 subjects were of sufficient diagnostic quality for measurement of mandibular cortical width. Five radiologist experts each measured

the cortical width in the mental foramen region of the dental panoramic radiographs from all recruiting centres (Figure 6.1). The cortical measurement site was located by use of a line passing through the mental foramen at right angles to a tangent at the lower border of the mandible. Measurements from both sides of the mandible were averaged and the mean value obtained from the observers was calculated for each radiograph. The radiographic measurements were corrected for magnification using a 3.175-mm-diameter ball-bearing positioned in the premolar region during the radiographic exposure. The ball-bearing acted as a reference object. Complete data from five observers were available for only 630 subjects. These were collected and analyzed in the regression model predicting mandibular cortical width.

Intra-oral radiographs were taken of the lower right premolar region, using an aluminium step wedge as a densitometric reference. The method of taking the radiographs was standardized in all centres using film-holding devices. Radiographs where there was insufficient bone available to make measurements, or which included obvious pathology, were not included in the analysis. It was possible to determine the jaw bone density from the radiographs of 526 subjects, using the mean pixel value for each reference step averaged over repeated measurements. The mean age of these 526 subjects was 55.1 yr (standard deviation (SD) = 6.2). In the other 137 subjects where it was not possible to measure the jaw-bone density, the mean age was 54.4 yr (SD = 5.5). There was no significant difference in age between the excluded and included patient groups ($t = 1.19$, $p = 0.23$). A region of interest was drawn interactively on the computer, covering the interdental bone of the right premolars. The mean gray value of the region of interest was measured using a software tool (Mevislab®, Mevis, Germany) and expressed as a corresponding thickness of aluminium (Figure 6.2). Data from 514 patients were analysed in the statistical model predicting AED (Al equivalent density).

### *Statistical analysis*

A regression model was used to investigate the association of the amount of alcohol consumption with the mandibular bone outcome variables, after adjusting for the subjects' age and menopausal status. In all cases, the residuals were

tested for normality. As a result of the stratified sampling used in one centre but not in the others, it was not possible to include a variable indicating recruitment centre in the regression analysis.

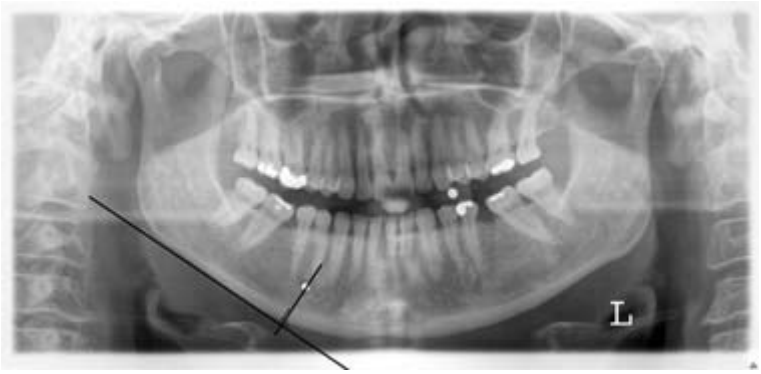



**Figure 6.1:** The mean cortical width was measured in the mental foramen region of the dental panoramic radiographs

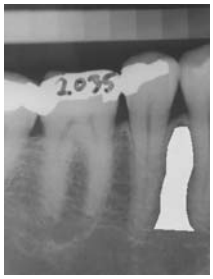


**Figure 6.2:** Intra-oral radiograph showing the region of interest for density measurement

## RESULTS

The mean age of the 663 subjects in the study was 55 yr (range 45–70; SD = 6). Of this study population, 660 subjects gave information about their alcohol consumption (Table 6.1). Full details of the numbers of patients participating in the analyses are given in Tables 6.2 and 6.3.

**Table 6.1:** Number of subjects (n) giving information on their alcohol consumption

| Alcohol consumption per week | n |
|---|---|
| Never consumed alcohol | 312 |
| 1-14 units consumed | 338 |
| > 14 units consumed | 10 |
| Total | 660 |

**Table 6.2:** Mean jaw-bone density related to alcohol consumption

| Alcohol consumption/wk | n | Mean (SD) AED |
|---|---|---|
| Never consumed alcohol | 261 | 5.70 (1.30) |
| 1 to 14 units | 255 | 5.43 (1.44) |
| > 14 units | 8 | 4.48 (1.94) |
| Total | 524 | 5.55 (1.39) |

**Table 6.3:** Mean cortical width (CW) related to alcohol consumption

| Alcohol consumption/wk | n | Mean (SD) CW (mm) |
|---|---|---|
| Never consumed alcohol | 301 | 3.72 (0.68) |
| 1 to 14 units | 331 | 3.42 (0.73) |
| >14 units | 10 | 3.51 (0.53) |
| Total | 642 | 3.56 (0.72) |

AED, Al equivalent density; SD, standard deviation

## *Mandibular bone density*

The mean aluminium equivalent for the sample was 5.55 mm (SD = 1.40). The concordance correlation coefficient for intrarater agreement for measurement of the EMD was shown to be 0.95 in a previously reported study. For inter-rater agreement, it was 0.87 (Nackaerts et al 2008). When age was the only independent variable in the model to predict EMD, the adjusted $R^2$ was 0.013, and with the addition of dummy variables for alcohol units consumed and for menopausal status, the adjusted $R^2$ was 0.023. The statistical model with subjects' age and alcohol units consumed per week was significant in predicting EMD, but explained only a minor amount of the total variance (2.3%).

For those consuming alcohol, either moderately or at greater amounts than recommended, the EMD was reduced compared with those who never consumed alcohol. The mean jaw bone density decreased with increasing alcohol consumption.

Table 6.4 shows the regression model with variables predicting mandibular bone density. The alcohol variables are dummy variables with reference category 'never consumed alcohol'; 'alc 1–14 units' represents consumption of 1–14 units of alcohol per week, and 'alc > 14 units' represents consumption of more than 14 units of alcohol per week. The variables representing menopausal status were compared with the premenopausal reference category. Both variables representing alcohol consumption were significant explanatory variables in the model ($p < 0.05$). The variable representing excessive consumption of alcohol (alc > 14 units) had a slightly greater standardized regression coefficient (-0.11) than

that for moderate consumption of alcohol (-0.09), indicating a more deleterious effect on mandibular bone density.

Multicollinearity occurs when there is a strong correlation between predictors in multiple regression. In the regression model, the collinearity statistical tolerance was > 0.2, indicating that there was independence between predictive variables, a necessary assumption in regression analysis. In addition, for the regression on EMD, the residuals were normally distributed with an equal spread of standardized residuals across the standardized predicted values. Five per cent of the standardized residuals had a value of > 2, indicating that the model was an acceptable representation of the data. The maximum Cook's distance was 0.19, which was below the critical value of 1. There was only one outlier data point that was more than 3 SDs from the regression line (i.e. the measured EMD values were 1.0 (predicted value 5.7)). Figure 6.3 indicates that the distribution of the residuals deviates only slightly from a normal distribution.

### *Mandibular cortical width*

The mean mandibular cortical width of the sample was 3.6 mm (SD = 0.72), measured from 645 panoramic radiographs. Intra-observer and interobserver variation data have been previously reported (Karayianni et al 2007). Interobserver repeatability was 2.15 mm for all five observers, as calculated using one-way analysis of variance (anova). The mean bias for the repeated measurements made by the same observer ranged between 0.37 and 0.23 mm.

When age was the only independent variable in the model to predict mandibular cortical width, the adjusted $R^2$ value was 0.10. Further addition of dummy variables for alcohol units consumed and for menopausal status resulted in an adjusted $R^2$ of 0.13, which was a significant increase ($p < 0.01$). Table 6.5 shows the regression model with variables predicting mandibular cortical width.

Comparison of the standardized regression coefficients for moderate alcohol consumption (1–14 units) showed that it contributed less to the model than the patient's age (-0.19 vs. -0.28). With excessive alcohol consumption, the standardized regression coefficient was -0.05, which was not significant ($p < 0.05$). The regression equation indicates that in female patients of the same age, those

who drink alcohol in moderation have a mandibular cortical width that is 0.27 mm thinner than those who have never consumed alcohol.

For the regression on cortical width, the multicollinearity statistical tolerance was > 0.2, indicating that there was independence between predictive variables. The residuals were normally distributed with an equal spread of standardized residuals across the standardized predicted values (Figure 6.4). Less than 5% of the standardized residuals had a value of > 2, indicating that the model was an acceptable representation of the data. The maximum Cook's distance was 0.10, below the critical value of 1. There were three outlier data points that were more than 3 SDs from the regression line: the measured cortical width values were 5.6 mm in each case, but the predicted values were 3.6 mm in each case.

**Table 6.4:** Regression model with variables predicting mandibular bone density

| | B | SE B | β |
|---|---|---|---|
| Constant | 7.06 | 0.64 | |
| Age | -0.02 | 0.13 | -0.10 |
| Alc 1-14 units | -0.26 | 0.12 | -0.09* |
| Alc >14 units | -1.27 | 0.53 | -0.11* |
| Postmenopausal | -0.17 | 0.22 | -0.06 |
| Perimenopausal | -0.21 | 0.21 | -0.06 |
| Irregular periods | 0.02 | 0.38 | 0.002 |

Alc, alcohol; B, unstandardized coefficient; SE, standard error; β, standardized coefficient

**Table 6.5:** Regression model with variables predicting mandibular cortical width

| | B | SE B | β |
|---|---|---|---|
| **Step 1** | | | |
| Constant | 5.64 | 0.248 | |
| Age | -0.04 | 0.004 | -0.32 |
| **Step 2** | | | |
| Constant | 5.50 | 0.29 | |
| Age | -0.03 | 0.01 | -0.28* |
| Alc-units 1-14 | -0.27 | 0.05 | -0.19* |
| Alcohol >14 | -0.28 | 0.23 | -0.05 |
| Postmenopausal | -0.03 | 0.10 | -0.02 |
| Perimenopausal | 0.04 | 0.09 | 0.03 |
| Irregular periods | 0.13 | 0.17 | 0.03 |

Alc, alcohol; B, unstandardized coefficient; SE, standard error; β, standardized coefficient

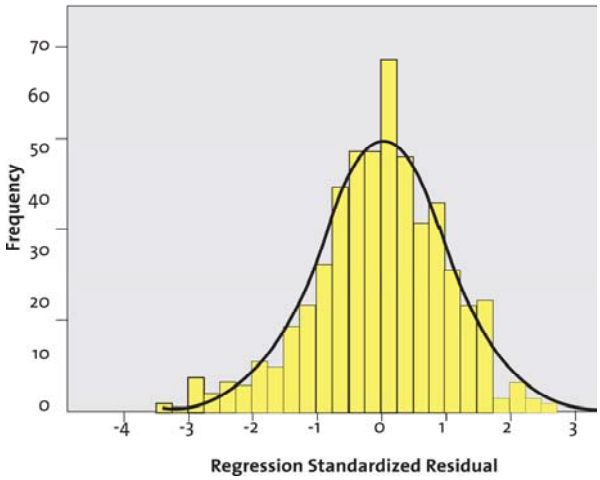



**Figure 6.3:** Distribution of the residuals across the predicted values for the regression on AED. The histogram shows only slight deviation from a normal distribution

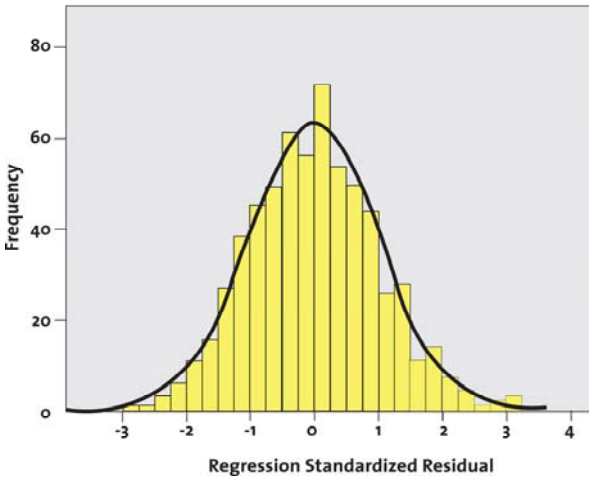



**Figure 6.4:** Distribution of the residuals across the predicted values for the regression on mandibular cortical width

## DISCUSSION

We have shown that in those who self-report moderate alcohol consumption, a reduced mandibular BMD and cortical width is seen. The statistical models were significant but accounted for minor amounts of variance, indicating that the association found may be statistically significant but of little clinical importance in healthy women. However, should the patient already have a thin, porous mandibular bone, additional risk factors, such as heavy alcohol consumption, may be of crucial clinical significance.

This large, cross-sectional European study is the first to demonstrate an association between alcohol consumption and mandibular cortical bone density and thickness. The frequency of alcohol consumption above the recommended levels is the factor most strongly associated with alcohol dependence (Dawson 1994). Future work will involve a more detailed comparison of mandibular bone density between those who are alcohol dependent and those who are not, involving the use of validated surveys such as the Alcohol Use Disorders Identification Test (AUDIT) (Bohn et al 1995). Although these detailed surveys are effective in identifying problem drinking, they are too time consuming to be completed by healthcare professionals in a primary-care setting. The health benefits of introducing screening tests and brief physician advice for patients who

drink alcohol excessively have been well documented (Fleming et al 2002), but there are additional considerations, such as the stigma associated with excessive alcohol consumption and the reluctance of healthcare professionals to confront the issue.

The influence of alcohol on skeletal BMD has been evaluated previously and shows a positive correlation between moderate alcohol consumption and mineral density (Felson et al 1995, Mukamal et al 2007), although excessive alcohol consumption is associated with increased fracture risk, especially hip fractures (Mukamal et al 2007, Kanis et al 2005). Excessive alcohol consumption and oestrogen deficiency are both risk factors for osteoporosis by altering the bone remodelling cycle and disturbing the balance of bone formation and resorption. Nevertheless, it seems that these factors act through different pathways. Although the exact mechanisms are still being investigated, in oestrogen deprivation, several cytokines (e.g. IL-6 and RANKL) that become more active due to this deprivation, accelerate the process of bone resorption. Consecutively, bone loss results when the bone formation rate cannot keep up with the bone resorption rate (Rosen 2006; Manolagas & Jilka 1995). The influence of alcohol is quite complex, due to its many secondary effects, e.g. through liver malfunction and malnutrition. The direct influence of ethanol on bone remodeling is to lower the osteoblast number and therefore reduce the bone formation rate. This is caused by endocrine modifications and increased OPG (osteoprotogerin) levels (Santorini et al 2008). To summarise, research evidence points to a higher bone turnover in oestrogen deficiency, where bone formation falls behind compared to bone resorption whereas the effect of excessive alcohol consumption is an inhibited remodeling. According to Callaci et al (2006), the 2 variables (oestrogen and alcohol) have independent and additive effects on bone loss. It is difficult to compare research findings on systemic bone with those found in alveolar bone because of many local factors influencing the jaw bone, such as oral hygiene and periodontal status. Further research could focus on the association of alcohol consumption with structural characteristics of alveolar trabecular bone.

The interobserver repeatability of manual cortical width measurements has been shown to be quite poor. In previous work, the difference between two measurements made by any pair of five observers for the same radiograph was

shown to be 2.15 mm for 95% of pairs of observations (Karayianni et al 2007). In the present study, the limited repeatability of measurements was mitigated by averaging the cortical width measurements of the five observers across each radiograph. Despite the poor reproducibility of measurements, both cortical width and densitometric measurements have been reported to be moderately correlated with BMD at other skeletal sites (Jacobs et al 1996, Devlin & Horner 2002).

To include an aluminium wedge on intra-oral radiographs is a challenge. A number of radiographs could not be used because teeth were overlapping the wedge or the wedge was partially or entirely out of the projection field. Moreover, radiographs were excluded when there was insufficient bone available to make the measurements, or which included obvious pathology. Lower exclusion rates can probably be reached when measuring at the level of the mandibular body.

From our study data (Tables 6.2 and 6.3) approximately half of patients said that they did not consume alcohol. This agrees with similar figures on the prevalence of alcohol consumption in those > 60 yr of age in the USA (National Institute for Alcohol Abuse and Alcoholism, 1997). Only a small proportion of subjects in our study (about 1.5%) stated that they consumed more than the recommended amounts of alcohol per week. In the 2006 National Survey on Drug Use & Health published by The Substance Abuse and Mental Health Services Administration (US Department of Health and Human Services 2008), 7.0% of 45–49 yr old subjects were heavy alcohol users, defined as drinking five or more drinks on the same occasion on each of 5 or more days in the past 30 d. This figure tends to decline with age; however, asking patients in a healthcare environment to self-assess their alcohol consumption may lead to underestimates of excessive consumption. Given the social humiliation associated with alcohol dependence, patients may have been reluctant to admit a high alcohol intake and this may have reduced the correlation with reduced mandibular BMD seen in our survey. In the present study we attempted to use a simplified measure of alcohol consumption with a threshold above and below 14 units per week, based on the recommendations of the Royal Colleges of Physicians, Psychiatrists and General Practitioners (1995).

Other clinical risk factors may affect mandibular cortical width and mandibular density (e.g. general risk factors for osteoporosis and fractures, such as genetic

factors, low body mass index, previous fractures, or corticosteroid treatment). Clinical risk factors from this data set have been reported in other publications (e.g. Devlin et al 2008).

The present study showed a reduced mean equivalent jaw bone density and mandibular cortical thickness in older individuals and in those with increased alcohol consumption. These factors have been known to affect bone density elsewhere in the skeleton, and therefore the mandible is not exempt from these systemic influences.

## ACKNOWLEDGEMENTS

This work was supported by a research and technological development project grant from the European Commission FP5 'Quality of Life and Management of Living Resources' (QLK6-2002-02243 OSTEODENT).

# Chapter 7

## Replacement therapy for periodontitis: pilot radiographic evaluation in a dog model

## INTRODUCTION

Beneficial bacteria have been extensively studied for their health-promoting effects (Parvez et al 2006). The main field of research has been in the gastrointestinal tract. In oral health, the beneficial effects on tooth decay have been reported for more than two decades (Herod 1991). In the past few years, probiotics have been investigated for periodontal health (Teughels et al 2008). These studies have shown that certain gut bacteria can exert beneficial effects in the oral cavity by inhibiting pathogenic species. The concept of periodontal replacement therapy, first proven by Teughels et al (2007), consists of applying beneficial oral bacteria subgingivally to prevent re-colonization of periodontal pockets by pathogens after scaling and root planing. The mechanism behind the concept seems to be related to bacterial interference and immune modulation. It is likely similar to the probiotic mechanisms of interaction in other parts of the alimentary tract. However, data are still sparse, and thus, more information is needed on the colonization of probiotics in the oral cavity and their possible effects on and within oral biofilms. Because of the globalization and increasing problems with antibiotic resistance, the alternative concept of probiotic therapy merits further research in the field of oral health care.

Detailed radiological analysis allows for *in vivo* detection of small density changes in the jaw bone (Jacobs et al 1996). Recently, a dedicated tool has been developed and validated enabling the detection of bone density changes as small as 6% in *in vitro* settings (Nackaerts et al 2006). Such subtle changes can be important to perform a controlled and standardized follow-up of bone re-modelling, on short term, which is not possible when visually evaluating two-dimensional images (Christgau et al 1998).

The aim of the current study was to radiologically evaluate the impact of replacement therapy by monitoring bone density changes and alveolar bone level (ABL) in periodontal pockets in a dog model. As such, not only the change in the vertical dimension of the alveolar bone but also its mineralization could be evaluated.

# MATERIALS & METHODS

## *Subjects*

Eight male beagle dogs suffering from mild periodontitis with an average age of 3.1 (± 0.4) years were used. The protocol was approved by the University's Ethical Committee for Animal Experimentation (K.U.Leuven, Belgium). Pockets were created surgically 4 months before the start of the study. With a water-cooled bur, 5mm of alveolar bone was removed from the canines, second, third and fourth premolars. The defects extended from mid-approximal to mid-buccal. Before wound closure, the root surfaces were conditioned with heparin (15,000 IU/ml) (Aventis Pharma, Brussels, Belgium) (Wikesjö et al 1991). All interventions were performed under general anaesthetics. Therapeutical procedures were described in detail previously (Teughels et al 2007). None of the dogs received antibiotics before or during the course of the study.

## *Interventions*

The pockets were randomly assigned to one of the following treatments by a clinician unfamiliar with the study design: Rp: Subgingival scaling and root planing at baseline; Bb: Subgingival scaling and root planing and repeated application of beneficial bacteria at baseline and weeks 1, 2 and 4.

The bacteria were described in detail by Teughels et al (2007). Pellets of Streptococcus sanguinis, Streptococcus salivarius and Streptococcus mitis were locally applied, pure and unsuspended, in the designated pockets by injection with a blunt needle. Pockets around teeth numbers 37, 38, 47 and 48 were assessed in the current study. Nine pockets belonged to the Rp group. Within the Bb group, application of single bacteria was done in 12 pockets (four pockets for each bacteria), and in 11 pockets, a mixture of the three bacteria was administered.

## *Radiography*

Radiographs were made before and after surgical pocket enhancement, at baseline (BL: 4 months post-operatively) and at week 12 (F: 7 months postoperatively). After anaesthesia, intra-oral radiography was performed with a Planmeca Prostyle Intra® device (Planmeca Oy, Helsinki, Finland) using

conventional F-speed film (Eastman Kodak Company, Rochester, NY, USA) and exposure protocol 70 kV, 8 mA, 0.24 s. Films were developed with an automatic film processor (XR 24 Nova®, Dürr Dental, Bietigheim-Bissingen, Germany) using fresh chemicals (Dürr Automat XR®) including automatic re-generation. The right and left premolar region of the lower jaw was radiographed. Rinn XCP® (Dentsply, York, PA, USA) film-holding instruments were adapted to contain an aluminium step wedge to standardize radiographs and to allow densitometric analysis. The wedge consisted of nine steps gradually increasing with 1.3 mm. At the time of creating the periodontal pockets, individualized impression moulds (Kerr Compound Sticks®, Kerr Corporation, Paris, France) were made for each dog and attached to a bite block to standardize the geometrical conditions of the radiographs. The use of the paralleling technique, complemented with a position holder, minimized image enlargement and geometric distortion of the radiographs. The conventional films were scanned at 800 dpi (Agfa SnapScan®, Agfa, Mortsel, Belgium).

## *Density*

For density analysis, custom made software was used, converting grey values into Al equivalent density (AED, in mm) values. The software was previously described and thoroughly tested *in vitro* (Nackaerts et al 2006). The region of interest covered the entire area where pockets were created (Figure 7.1).

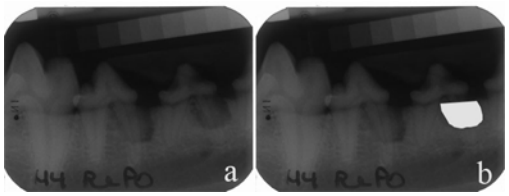



**Figure 7.1:** a) Original radiograph; b) Delineation of the region of interest, covering the entire pocket area

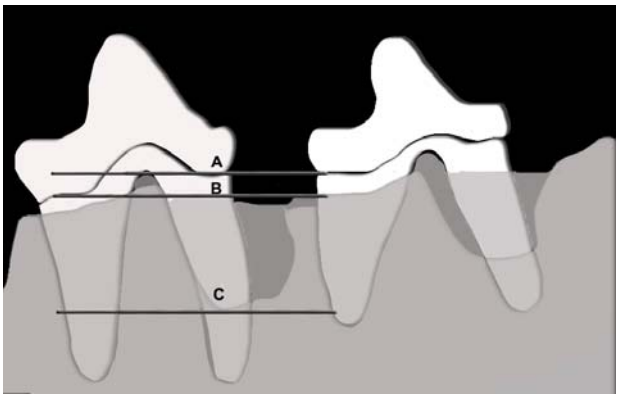



**Figure 7.2:** Change in bone level was assessed by measuring distance BC. The distance AB was used for correcting a potential generalized change in horizontal alveolar bone level

### *ABL*

ABL was measured in Adobe Photoshop® (Adobe Systems Incorporated, San Jose CA, USA). The width of the step wedge was used as a correction factor for potential vertical distortions. The facing cemento-enamel junctions of teeth 37 and 38 (for measuring bone level at pocket round tooth 38) and between 36 and 37 (tooth 37) were connected (line A; Figure 7.2). The same was done for teeth 47 and 48 (tooth 48), and 46 and 47 (tooth 47). Line A served as a reference for all further bone-level measures. A second line was drawn through the highest point of the inter-proximal bone crest, parallel to the first line (line B; Figure 7.2). Another line, parallel to the first, was drawn contingent to the lower border of the surgically created pocket (line C; Figure 7.2). The distance A to B was considered as a reference to explain the observed bonelevel change over time. If it would be registered as increasing over time, a decrease in total crater depth could be attributed to this, while it should actually be qualified as bone loss. If on the other hand |AB| would remain stable, any observed change could be attributed to a change in the base of the crater. This strategy allowed a critical assessment of the observed bone level changes.

### *Statistical analysis*

Because of a limited number of conditions and measurements, descriptive statistics were applied to attempt finding distinct healing in the Rp and Bb group. With the pocket as the unit of analysis, the Wilcoxon test for paired samples was used to detect differences in measurements at baseline and 12 weeks later.

## RESULTS

Not all radiographs could be analysed. Because of inadequate image quality caused by movement during exposure (8 sites) and the loss of radiographs during development (4 sites), only 20 sites were available for analysis. Out of these sites, 8 pockets belonged to the Rp group and 12 to the Bb group. Out of these 12, in 5 the mixture of bacteria was administered, and in 7 a single bacteria (two: S. mitis, four: S. salivarius, one: S. sanguinis). The measurements of the Bb group were pooled.

Table 7.1 gives an overview of the Wilcoxon test for measurements of the density of the pocket area and radiographically evaluated ABL. No significant differences in radiological measurements (n = 8) could be found between baseline and week 12 in the Rp group. In contrast, differences in density and ALB measurements yielded statistical significance for the Bb group (n = 12).

Starting from the baseline images, the percentage change in bone density and bone level was visualized for the two treatment modalities. Figures 7.3 and 7.4 show the results. Density measurements showed a decrease in density for Rp and an increase for Bb 12 weeks from baseline. In Figure 7.4, the gain in bone level is shown to be higher in the Bb group.

**Table 7.1:** Wilcoxon test for density of the pocket area and radiographically evaluated alveolar bone level (ABL)

| **Density** | **Rootplaning** | | **Benificial bacteria** | |
|---|---|---|---|---|
| | **BL** | **F** | **BL** | **F** |
| n | 8 | 8 | 12 | 12 |
| Median | 5.85 | 5.80 | 5.40 | 5.70 |
| Interquartile range | 5.25 - 6.55 | 5.20 - 6.80 | 5.05 - 6.30 | 5.40 - 6.85 |
| p-value | | 0.95 | | 0.03 |
| **ABL** | **Rootplaning** | | **Benificial bacteria** | |
| | **BL** | **F** | **BL** | **F** |
| n | 8 | 8 | 12 | 12 |
| Median | 0.47 | 0.41 | 0.45 | 0.35 |
| Interquartile range | 0.46 - 0.50 | 0.32 - 0.51 | 0.39 - 0.48 | 0.32 - 0.45 |
| p-value | | 0.25 | | 0.04 |

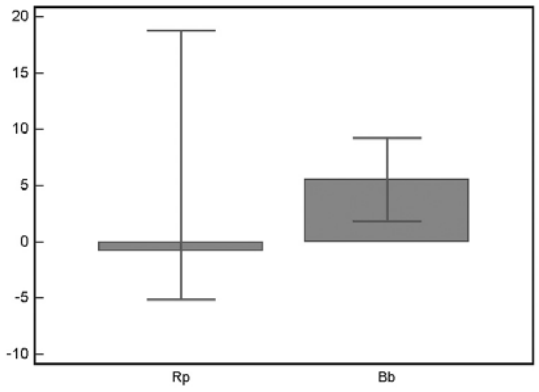



**Figure 7.3:** Percentage of bone density change in the pocket area between baseline and 12 weeks after therapy

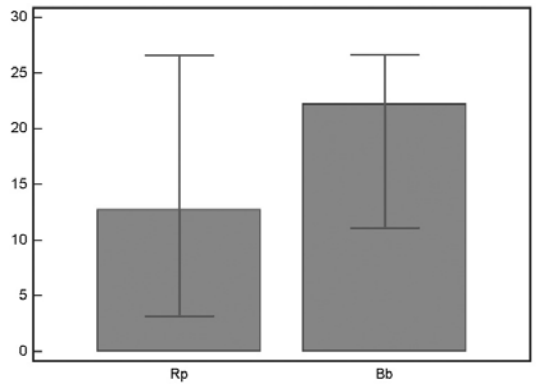



**Figure 7.4:** Percentage change in bone level between baseline and 12 weeks after therapy

## DISCUSSION

When evaluating bone healing after periodontal replacement therapy, differences between the control periodontal pockets and the pockets receiving beneficial bacteria were recorded. Significant improvement in jaw bone density and alveolar bone gain was found in periodontal pockets that received beneficial bacteria adjunctive to scaling and root planing. Such significant differences were not found in periodontal pockets that received only scaling and root planing. It was previously confirmed that in pockets treated with beneficial bacteria, subgingival re-colonization of periodontopathogens was delayed and reduced, as was the degree of inflammation (Teughels et al 2007). Based on our results, radiologically, the healing of a periodontal pocket after scaling and root planing seems better when beneficial bacteria are applied.

One should consider the data obtained in this pilot study as preliminary. It is evident that it is impossible to draw definite conclusions taking into account the shortcomings involved in the present study. If the animal (i.c. beagle dog) would be the unit of analysis, a confounding factor might be the inter-subject variation. To handle this shortcoming, a split-mouth design was chosen. Yet, it is clear that the latter is also far from ideal. Because of ethical considerations, the number of animals to be used is limited, resulting in a small sample size with less persuasive results. In addition, it cannot be excluded in such design that intra-oral translocation takes place (Hujoel & DeRouen 1992), which in turn could distort the outcome. Considering these factors, data for single and mixed application of beneficial species were pooled in the frame of this pilot study. The results can therefore only give an idea of the potential effect of the application of probiotics on bone regeneration as assessed on periapical radiographs; definite conclusions on the effect of specific species and/or combined treatment need further studies with a larger sample size enabling a more robust comparison of different therapies.

As to the knowledge of the authors, no study has ever followed up jaw bone density changes as a result of periodontal therapy over such a short time. Consequently, the differences that were found are small. It is known that to visually detect a change in bone density on consecutive radiographs, a rather large amount of change in mineralization needs to occur (Southard & Southard 1994, Christgau et al 1998). However, thanks to computer-aided densitometric

image analysis, the detection of rather subtle bone changes has become feasible (Brägger 2005). Even after 6 months, Eickholz et al (2007) could only find non-significant differences after guided tissue regeneration in infrabony defects. Holland et al (1998) found that 12 months after removal of silk ligatures inducing periodontal disease in a dog model, the alveolar bone had increased in height. Linear alveolar bone measurement at 9 months revealed no such difference. The pockets created in the current study are to be defined as wide, rather than deep and small. Eickholz et al (2004) found a more pronounced healing of infrabony defects that were narrow and deep in comparison with shallow but broad defects. In addition, it is possible that bone growth at the base of the crater was underestimated, due to insufficient maturation for the bone to be visible on the periapical radiographs (Yun et al 2005).

Radiographic assessment of jaw bone is associated with sources of error, such as exposure settings, geometry, development of films, etc. Many of these were addressed in the study set-up. The step wedge did not only offer the possibility of standardizing density (Nackaerts et al 2007) but also enabled taking into account geometric distortion of the image. Furthermore, the stent reduced the chance of projection errors in this follow-up approach. Another factor potentially introducing errors is the use of conventional films rather than digital image plates. Borg et al (2000) assessed the marginal bone level around implants in a dog study and found no difference in accuracy and precision between digital and conventional films. This was confirmed by Pecoraro et al (2005). Nevertheless, Li et al (2007) found that digital films, corrected for attenuation and visual response, did improve the measurement accuracy. In the current study, it was not feasible to use digital films, though this should be taken into account in future study setups. An additional benefit of digital radiography is the possibility of immediate retakes in case of image errors, although this is not applicable in patient studies. One more specific problem related to this radiographic study in a dog model includes the stabilization of the beam-aiming device. Involuntary tics could shift the aiming device during exposure. Therefore, some of the radiographs needed to be excluded for analysis.

The current study set-up enabled the *in vivo* evaluation of bone density and height, using standardized imaging, creating a versatile tool to monitor minute

changes in the jaw bone. Despite the limitations mentioned, this made the study unique in animal research.

In conclusion, this pilot study indicates the potential effect of a subgingival application of beneficial species in periodontal pockets on bone healing and illustrates the strength of follow-up radiography to evaluate the effects of different treatment strategies on bone re-modelling. The integrated use of jaw bone density evaluation and alveolar bone height measures is assumed ideal in animal models to test new treatment or surgical strategies.

## ACKNOWLEDGEMENTS

This study was supported by NIDCR grant DE015360 (M. Quirynen). The authors wish to thank L. Corpas for drawing Figure 7.2.

# Chapter 8

## Density and structure of the jaw bone assessed in 2D and 3D radiography

## INTRODUCTION

Jaw bone quality assessment has relevance in many fields of dentistry: implant dentistry, endodontics, periodontology and maxillofacial surgery. Indeed, information on bone quality may influence diagnosis and treatment decisions on planning and follow-up (Huumonen et al 2006, Iqbal & Kim 2008, Ong et al 2008). Radiology is the clinical method of choice for assessing bone quality, given the continuous development and refinement of image analysis modalities in research. Despite this, the daily interpretation of radiographs by dental professionals has still remained subjective and not statistically validated to a large extent. A tool for bone assessment could assist in making evidence-based clinical decisions.

Intra-oral radiographs are used routinely in the dentist's office; however, levels of observer variability in diagnosis are high and image analysis techniques may offer the possibility of more objective and accurate diagnosis. In addition, the quality of radiographs in general dental practice is frequently sub-optimal and image analysis may provide a means of extracting information that cannot be discerned visually. We previously developed a tool for bone quality assessment on intra-oral radiographs (Nackaerts et al 2006). This tool was validated, though it deserves further attention on how the measurements reflect the actual characteristics of the bone on a microscopic level. CBCT use for dentomaxillofacial applications is rapidly proliferating. It provides high quality three-dimensional images at a relatively low radiation dose (Loubele et al 2008), justifying its use for many applications, considering the added value of three dimensions by providing information to facilitate diagnosis and therapy (Jacobs et al 1999). Even this high level imaging tool is not equipped with an intrinsic method for quantifying bone characteristics. The typical CT-based Hounsfield scores that are traditionally known as a relative indicator for jaw bone density, do not apply in dental CBCT (Yamashina et al 2008).

The aim of this *in vitro* study was therefore to assess clinically applicable radiographic methods for objective bone quality evaluation. The investigated features were density and structural assessment of jaw bone in periapical radiographs as well as on CBCT slices. Morphometry on microscopic images of the bone served as a reference standard.

## MATERIALS & METHODS

Twenty four dry human bone samples from the mandibular premolar region were obtained from the Department of Anatomy of the KU Leuven with ethical approval (Commission of Medical Ethics, University Hospitals of the KU Leuven). All samples were blocks cut from different mandibles, with an intact cortical bone plate. The mean height of the samples (mandibular base to alveolar ridge) was 25.33 mm (SD = 4.24) and the mean length (distance between cutting surfaces) 11.23 mm (SD = 2.28).

### *Radiography*

Periapical radiographs were made with the Minray® intra-oral device (Soredex, Tuusula, Finland) using the following exposure parameters: 7 mA, 70 kV and 0.12 s. Normal clinical practice of periapical radiography was simulated, with the buccal face of the samples facing the X-ray source and the image receptor in close apposition to the lingual surface of the bone samples. Image receptors were Dürr phosphor plates, scanned with Dürr Vistascan® (Dürr, Bietigheim-Bissigen, Germany). Together with the samples, an aluminium wedge with 9 steps increasing in thickness by 1.3 mm was radiographed, serving as a densitometric reference (Nackaerts et al 2006).

For the purpose of CBCT scanning, the samples were placed on a polystyrene foam surface, fixed on an aluminium platform. They were positioned with the mesial cutting surface on the polystyrene foam. Scans were made with the Scanora 3D® device (Soredex, Tuusula, Finland) using the following exposure protocol: 85kV, small field of view (60 x 60 mm), high resolution, 8 mA with a scan time of 20 s. The voxel size was 0.13 mm.

### *Microscopy*

The distal surface of the samples was examined with a Nikon 80i® light microscope (Nikon, Chiyoda-ku, Tokyo) with a magnification of 40. The images were analysed using the Lnet software (Nikon).

## *Image analysis*

For each bone sample and each evaluation method, one and the same side was analysed. The middle 8 mm of each image was selected (Figure 8.1). As such, the output data could be compared in a sensible way and potentially correlated.

In the periapical radiographs, a square of 1 mm x 8 mm was selected at the distal surface. On this square, aluminium equivalent density (AED) was measured using the methodology and custom software previously described (Nackaerts et al 2006).

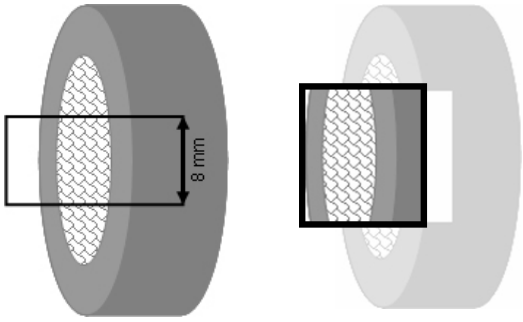



**Figure 8.1:** Region of interest for all imaging methods. The middle 8 mm was selected. Density on intra-oral radiographs was measured on this 8 mm section, over a width of 1 mm. Two CBCT slices for this 8 mm height were analysed, covering 230 µm of analysis width. The microscopic image was analysed with a focus depth of 150 µm.

Two CBCT slices (Figure 8.2) from the distal surface were processed for image analysis in ImageJ (public domain, NIH, Maryland, USA). First, the slices were imported and a region of interest (middle 8 mm as shown in Figure 8.2) was cropped. Within this region, the trabecular bone area was manually selected. This trabecular bone image was made binary with the automated isodata algorithm for thresholding. The algorithm initially segments the histogram into two parts using a starting threshold value: half the maximum dynamic range. The sample mean of the gray values associated with the foreground pixels and the sample mean of the gray values associated with the background pixels are computed. A new threshold value is then computed as the average of these two sample means. The process is repeated, based upon the new threshold, until the threshold value does not change any more. The bone area fraction was calculated as the percentage of white pixels in the binarised image of the trabecular bone area. The bone area fraction was averaged for the slices for each sample.

On the microscopic images (Figure 8.2), measurements were done on one side, with a focus depth of 150 µm. The total width and cortical width of the 24

samples were calculated. This allowed analysis of whether aluminium equivalent density could be used in characterization of the bucco-lingual bone width, including the trabecular fraction of the samples, or whether it rather characterised the cortical width. Five horizontal lines were drawn from the buccal to the lingual cortical border and their length was averaged. The first line was drawn 4 mm superior to the sample midline; the other 4 lines were drawn below the first with 2 mm spacing. The cortical width was traced out on the same 5 lines and averaged. Bone area fraction was based on intercept thickness measurements using the following method: A grid, formed of 7 horizontal and 6 vertical lines drawn at 1 mm intervals covered the trabecular bone area. The bone components on these lines were counted and expressed as a percentage of the entire length of all lines. This percentage was called bone area fraction for reporting the results. Test-retest reliability for this method was excellent (concordance correlation coefficient for repeated measurement = 0.87).

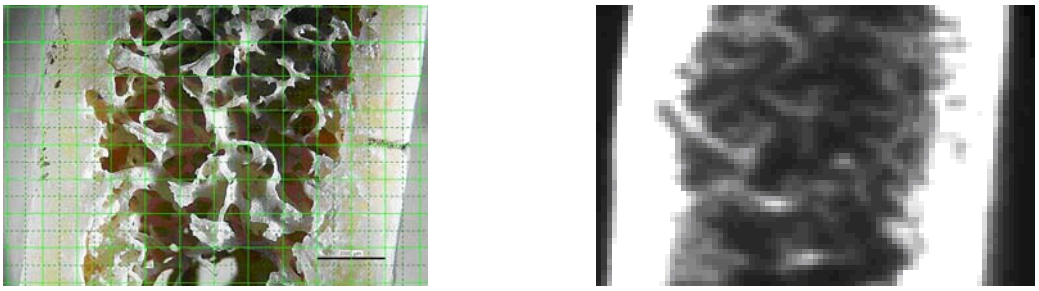


**Figure 8.2:** Illustration of imaging modalities showing the region of interest of one sample (8 mm height). a) Microscopic image showing the grid placed on the region of interest; b) CBCT slice

### *Statistical analysis*

Correlation analyses were performed for the measured variables. Pearson's correlation coefficients were used after normality was confirmed using d'Agostino-Pearson test.

## RESULTS

Sample properties are described in Table 8.1.

**Table 8.1:** Sample characteristics (n = 24)

| Variable | Mean | SD |
|---|---|---|
| **Periapical RX** | | |
| AED[a] (mm) | 4.36 | 1.35 |
| **CBCT[b]** | | |
| Bone area fraction (%) | 21.05 | 10.87 |
| **Microscopy** | | |
| Sample width (µm) | 9496 | 1756 |
| Cortical width (µm) | 3531 | 726 |
| Bone area fraction (%) | 20.35 | 9.28 |

[a]AED, aluminium equivalent density; [b]CBCT, cone beam CT

There was a highly significant positive correlation (Pearson's r = 0.67; $p < 0.05$) between the measurements on the microscopic images and CBCT slices. The scatter plot for this correlation is shown in Figure 8.3.

The correlation Table (8.2) for AED with the variables measured on microscopic images shows a strong positive correlation for all microscopy measurements. The correlation coefficient was highest for the cortical width (Figure 8.4), but all coefficients were above 0.60.

**Table 8.2:** Correlation table (Pearson coefficients) for measurements on microscopic images and AED (aluminium equivalent density)*

| | Sample width | Cortical width | Bone area fraction microscopy |
|---|---|---|---|
| **AED** | 0.65 | 0.79 | 0.61 |

*$p < 0.05$ for all r

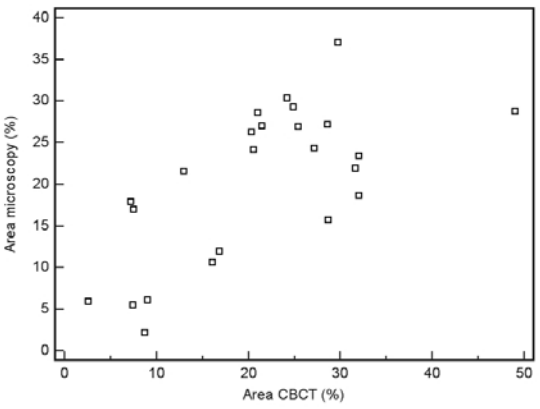



**Figure 8.3:** Scatter plot for the bone area determined on CBCT slices versus the bone area determined on microscopic images of the bone

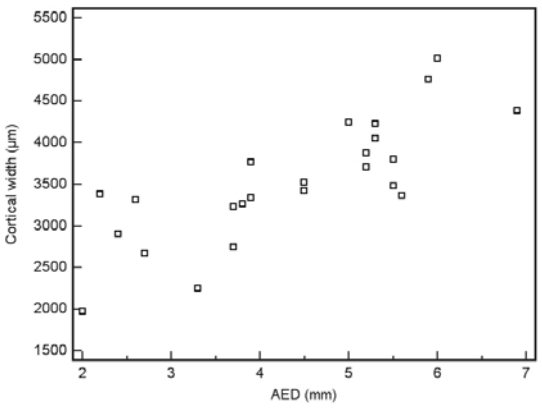



**Figure 8.4:** Scatter plot of the aluminium equivalent density (AED) and cortical width

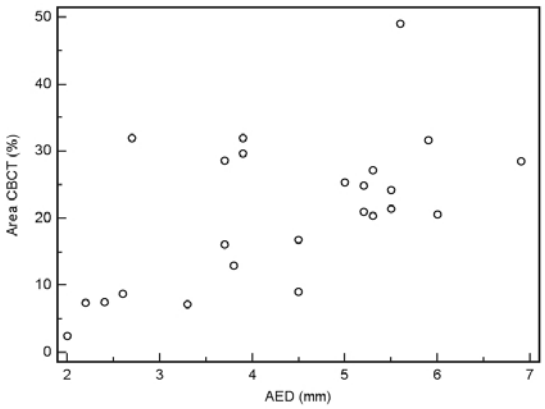



**Figure 8.4:** Scatter plot of the aluminium equivalent density (AED) and Bone area fraction measured on CBCT images

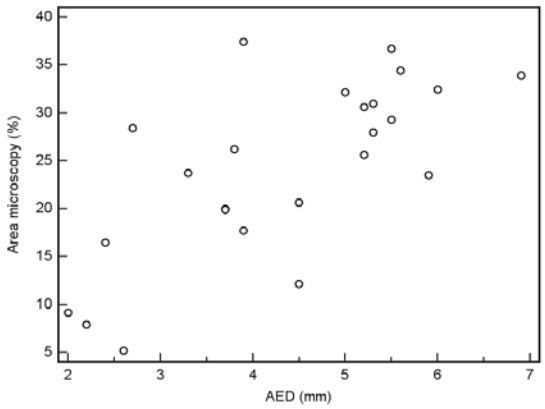



**Figure 8.5:** Scatter plot of the aluminium equivalent density (AED) and Bone area fraction measured on microscopic images

## DISCUSSION

Bone quality evaluation based on two frequently used imaging modalities in dentistry was tested against a reference standard of microscopic measurements. Structural measurements on CBCT images were significantly correlated to the structural measurements on microscopic images. AED was a strong predictor for cortical thickness of the mandibular bone samples used in the current study.

We did not evaluate structural information on the intra-oral radiographs. In CBCT, the separate slices allow direct structural measurements, as opposed to measuring the overlapping structures in a two-dimensional intra-oral image. Moreover, in the periapical radiographs, the overlapping cortical border causes a further blurring of the structure, impeding the segmentation procedure. Nevertheless, Apostol et al (2006), confirmed that textural analysis of two-dimensional images could predict up to 93% of the variance in three-dimensional micro-architectural properties of bone. Although their experimental set-up was not yet applicable in clinical practice (µCT), their approach is promising, e.g. in refining osteoporosis diagnosis. The influence of the cortical thickness on AED was expected, since it was previously described that a large amount of trabecular bone can be removed before it is detectable on a radiograph, while the involvement of cortical bone in the deleterious process is more rapidly visualised (van der Stelt 1985).

Previous research showed the validity of µCT images as an alternative for histological slicing, being time consuming and destructive (Stoppie et al 2005). Although certainly applicable *in vitro* and in animal studies, µCT is still not suitable for clinical applications due to the associated long scan time, high radiation levels and limited field of view. The results of the current tentative research suggest that CBCT images could provide a clinically applicable method for accurate structural characterisation of jaw bone. This finding should be verified in further studies, introducing possible confounders of the strong correlation, such as soft tissue and movement artefacts.

Dry bone samples were used, making a generalization to clinical applications not yet possible. The presence of other objects, i.c. an entire mandible or skull, influences the density results of the object of interest (Katsumata et al 2007). Possibly this influence alters the structural analysis e.g. through differences in segmentation. Moreover, the presence of water or soft tissue equivalent would have increased the noise in the image (Weldon et al 2008). Therefore, surrounding the sample with soft tissue equivalent might have yielded different correlations in CBCT as well as in periapical images.

A good correspondence was found between structural parameters in CBCT slices and a microscopic image of the sample surface, providing an overall structural reference value of the sample. This result calls for further validation, which can be reached through matching histological slices to the equivalent CBCT slices (Stoppie et al 2005). The results of such registration procedure could reveal the actual potential of CBCT in non-destructive highly accurate bone quality evaluation.

Because of the routine use of intra-oral radiographs and the increasing application of CBCT imaging, it could be worth developing reference values based on this type of image. There is in fact a need for validated, clinical methods for jaw bone quality assessment, for example prior to implant surgery (Ribeiro-Rotta et al 2007).

It was confirmed in this study that, whenever information on the three-dimensional structure of jaw bone is needed, analysis of three-dimensional images is preferable. To obtain an overall objective evaluation of bone quality, intra-oral radiographs may be used.

# Chapter 9

## General discussion and conclusions

There is a need for an objective clinical method of measuring bone quality before oral surgical procedures such as implant placement. In the absence of a readily available clinical device that can evaluate and measure objectively jaw bone quality, the work presented in this thesis had the overriding aim of developing a simple measurement tool and a bone quality assessment strategy. In addition, the work in this thesis attempted to identify those imaging techniques and methods of analysis that could link bone quality to other parameters, such as systemic bone density, periodontal bone loss and bone architecture.

## METHODOLOGY

### *Oral Imaging Methods*

The oral imaging methods that were used in this thesis were intra-oral radiographs, panoramic radiographs, and CBCT. Intra-oral radiographs are common imaging modalities used in almost all dental practices, whereas panoramic radiographs are used less frequently, in about half of practices. CBCT is a newer and more advanced imaging modality that is found in only a few dental practices and which has more specific indications. In any case, all have benefits and drawbacks and, with respect to the ALARA principle, the imaging modality to be used should be selected based on the diagnostic requirements combined with the least possible radiation dose.

Panoramic radiographs are often used as an initial evaluation tool, e.g. in orthodontics, periodontology or for the assessment of 3rd molars. Based on these images, clinicians may decide on the need for further examinations. Despite the lack of evidence to support panoramic radiography as a “screening” tool, this practice is common (Rushton & Horner 1996, Rushton et al 2001). Amongst the disadvantages of panoramic images is the inability to display fine anatomic detail. The reason for this is not only the 2D projection of 3D structures, but also the magnification and geometrical distortion of the image. Furthermore, the spatial resolution is not as high as intra-oral radiographs. This hampers the detection of minor or nascent lesions. When using panoramic radiographs in our research to assess bone density and alveolar bone level (Chapter 2) we came across some of these drawbacks, leading to substantial loss of precision in the measurements.

Certainly, panoramic radiography is useful in clinical practice, especially in terms of patient comfort for initial treatment planning. The detection and/or large deviations of important anatomical structures is also possible. For research purposes, it seems that panoramic radiographs are useful when performing repeatable and standardised measures, such as assessing cortical width (Ledgerton et al 1999, Devlin et al 2007c). Also, more advanced techniques, such as active shape modelling (Allen et al 2007) and fractal analysis (Geraets et al 2007) certainly offer potential since they are less dependent on the shortcomings of the panoramic technique. These advanced techniques will continue to be a topic of research.

Intra-oral radiographs have the advantage of high spatial resolution and, with the optimal exposure settings, a vast amount of contrast information. Thanks to digitalisation, even more information can be recorded on the image receptors. Intra-oral radiographs have proven to be useful in periodontal bone assessment, both in assessing the alveolar crest height and alveolar density changes. Also, they can be a useful tool in the follow-up assessment of endosseous implants. In the *in vivo* studies, (Chapters 5 and 6) we did not use digital image receptors. If, however, a digital system had been used, there would have been a larger span of available gray values for analysis, meaning an increased contrast resolution and a loss of films due to processing errors would have been prevented. To scan conventional radiographs possibly coincides with a loss of information (Hangiandreou et al 1998). According to Parissis et al (2005), the digitized counterparts of conventional radiographs show higher density values and a reduced dynamic range, visible as increased contrast. Nonetheless, this increased density was proportional, which makes the investigated relationships in our studies still valuable. The latest imaging modality described is CBCT for dental applications. It offers a great potential for the future since it enables the visualisation of three dimensions, and removes the most important drawback of the previously mentioned techniques (Chapter 9, Yan et al 2009). Superimpositions are eliminated through the reconstruction process of the original projections. The study performed in the framework of this thesis was an exploration of the potential of CBCT images for jaw bone characterization,

although this will inevitably require more in depth research and, at a later stage, a clinical evaluation.

The relatively low cost of CBCT compared with conventional computed tomography systems, and the substantially lower relative dose, (Loubele et al 2008) has led to the rapid emergence of CBCT systems in just a few years (Guerrero et al 2006; www.sedentexCT.eu). It is an ideal source of information for implant planning, but also other surgical or dental procedures, such as root canal treatments and surgical removal of impacted canines. In oral implant planning, it is important to know local bone quality to guarantee successful healing and osseointegration. Furthermore, the planning procedure of implants includes the reconciliation of implant dimensions, esthetics and biomechanics with the limitations of anatomic dimensions, morphology and neurovascularisation. CBCT images give access to a great information pool to explore, not as a screening tool but for local bone diagnostic tasks.

There are still drawbacks of CBCT compared to conventional (multi slice) spiral computed tomography. The reconstructed images cannot be interpreted in the same way, due to the inhomogeneous properties of the beam and receptor used, even varying between devices. This hampers an unequivocal assessment of bone quality and the identification of normal and deviating values. Hounsfield units (HU) are used in conventional CT images for the density assessment of, among other tissues, bone (Shapurian et al 2006). The HU are expressed relative to air (-2000), water (0), and dense bone (+2000). These values are not applicable in CBCT due to the intensity inhomogeneity of the beam and the associated low contrast resolution. It should still be possible to define a quantitative tool, comparable with HU. There are several technical aspects to be resolved before such CBCT density value would be applicable: the exact relationship between pixel values of bone structures and the actual properties of these structures should be known; whether the resolution over the entire scanned volume is homogeneous and if it can be mathematically described. CT permits the resolution of objects which differ only slightly in their attenuation of radiation, while this low contrast resolution is inferior in CBCT. The imaging of soft tissues and their pathology is therefore not possible in the latter. Quantitative computed tomography can provide bone mineral density (BMD) information by relating density information from tomographic scans to a

calibration phantom with predetermined BMD. That is not possible for CBCT, because of the previously mentioned intensity inhomogeneity. This drawback might be solved in the future, but will require equipment-dependent solutions, since CBCT devices have diverse operating mechanisms.

Other possible imaging modalities for the characterisation of jaw bone are ultrasound (US), magnetic resonance imaging (MRI) and DXA. Haffar et al (2006) studied the feasibility of using ultrasound quantitative measurements to identify jawbone quality preceding implant placement. They used speed of sound (SOS) measurements at several regions of the mandible and found it to be strongly correlated to BMD of these regions as measured with DXA, and also to trabecular to cortical bone thickness ratio. The obvious advantage of quantitative ultrasound is the absence of ionizing radiation. The portability of the tool and its low cost make it suitable for clinical use. Nevertheless, information on the relation of speed of sound and various bone properties needs to be further specified. Another tool that does not involve ionising radiation is MRI. It was successfully applied for trabecular bone quality evaluation (Choël et al 2004, Celenk & Celenk 2008), but cannot be considered as a clinical tool for routine use, at least not in the near future. The same goes for DXA. It is considered to be the technique of choice for assessment of BMD in central and peripheral skeletal sites and has been applied for jaw density as well (Stoppie et al 2006, Devlin et al 2007b, Drage et al 2007), but until now merely in experimental settings. There is no software available for jaw bone BMD measurements. Forearm or small animal software has been reported for these assessments.

### *In vitro studies*

For the *in vitro* studies, we decided to work with samples cut from the premolar mandibular area of cadavers. The samples were kept in their original shape, meaning that they spanned the entire mandibular ridge, from the mandibular lower border to the alveolar crest. This allowed a closer approach to the *in vivo* condition than cutting out cubic or cylindric bone samples of predetermined size, as is often done. In some research, the cortical border of the samples is removed for better structural characterisation (Stoppie et al 2006). This type of sample preparation allows the control of many variables, but does not resemble true jaw bone

visualised in the clinical situation. The influence of the cortical bone in detecting jaw bone density and structure was exactly one of the topics we did not wish to ignore. Related to this is the use of soft tissue or soft tissue simulation. In the *in vitro* set-up we used in our studies, there was no soft tissue simulator present when taking radiographs, although the presence of fat and muscle tissue would have influenced the density results due to an altered attenuation of the x-rays (Souza et al 2004). However, since the set-up was highly standardised, the addition of identical soft tissue simulator in all samples would not have changed the relations found. The use of formalin fixed jaws would have been possible but formalin has a demineralising effect on bone tissue (Fonseca et al 2008), which would not have been reconcilable with the deliberate demineralisation during the precision studies. This does not change the fact that in bone density assessments in patients, the influence of soft tissue can be a confounding factor (Souza et al 2004).

### *Dog model*

Based on the results of chapter 7, radiologically, the healing of a periodontal pocket after scaling and root planing seems better when beneficial bacteria are applied. If the animal (i.c. beagle dog) would be the unit of analysis, a confounding factor might be the inter-subject variation. To handle this shortcoming, a split-mouth design was chosen. Yet, it is clear that the latter is also far from ideal. Because of ethical considerations, the number of animals to be used is limited, resulting in a small sample size with less persuasive results. In addition, it cannot be excluded in such a design that intra-oral translocation takes place (Hujoel & DeRouen 1992), which in turn could distort the outcome.

Another specific problem related to this radiographic study in a dog model includes the stabilization of the beam-aiming device. Involuntary tics could shift the aiming device during exposure. Therefore, some of the radiographs needed to be excluded for analysis.

## CLINICAL IMPLICATIONS

About 98% of dentists in Belgium have direct access to intra-oral radiographic equipment and intra-oral radiographs amount up to 20% of medical radiographic

images taken each day. As opposed to general medicine, where ionizing radiation is only used by radiologists, general dentists fulfil this role on a daily basis. This means there is a huge amount of information on teeth and jaw bone available to them, most of which is not used to its full extent. The situation could be different if clinical tools, such as the one developed in the framework of this thesis, were incorporated in the image analysis software associated with the radiographic equipment. Moreover, if normal values were available on jaw bone characteristics, screening of jaw bone quality would be feasible, indicating the state of the bone compared to bone of age-matched subjects. These normal values could be obtained from large scale clinical studies. For true comparative data, certain fixed parameters should be defined, such as gender, age and anatomical site of the jaw.

Absolute quantification of bone density values requires the use of a calibration wedge of known density and dimensions, a method known in systemic bone density measurements (Matteson et al 1996). Although the use of the wedge as described in the thesis might not be feasible in the general dentist office, a modified wedge could resolve this practical issue. There is more room for improvement, namely in the inclusion of the reference wedge in the aiming device. This wedge should be large enough for valid analysis, but interfere in the least possible way with convenience for the patient and the clinician. The anatomical coverage available for interpretation should not be reduced. It should be feasible, certainly now that phosphor plates are more regularly used, to include a similar reference material. However, for direct digital devices (Coupled Charge Device, CCD), the active area is essentially smaller, jeopardizing optimal visualisation of the region of interest. The comfort of the patient is also important. For some patients it is more difficult to support the edges of the image receptor (Berkhout et al 2002). If measurements or assessment of the basal bone, rather than alveolar bone or teeth, are to be carried out, this becomes truly relevant (Lindh et al 2008). To have a range of 3 known densities within the range of true bone density could suffice for the calibration purpose. Inclusion of this reference into the image receptor itself might be an option. Besides general screening of jaw bone quality, specific applications gain benefit from density quantification. The use of image calibration, in individual images but also in subtracted consecutive images of the same subjects has proven its use in studies concerning the detection of

periodontal and periapical defects and the healing of those defects through the detection of subtle density changes. The success of implants has some dependence on bone density (Jaffin & Bernen 1991). In orthodontics, when the use of miniscrews is considered, density information is also important, since the rate of tooth movement depends on bone density (Park et al 2008). But even then, it should always be kept in mind that radiographic parameters must be integrated with patient history and clinical observations to come to a final diagnosis.

In conclusion, standardised bone quality evaluation has several potential clinical applications, but the idea of a single ideal measurement unit does not seem feasible. Instead, a combination of analyses on the same image as well as the inclusion of clinical parameters such as age and gender, could improve the diagnostic value to a large extent. Intra-oral radiographs would be a suitable source for such a combined approach, e.g. density and fractal dimension assessment, including age information. For panoramic radiographs, this approach has shown to be successful in osteoporosis screening (Devlin et al 2008, cfr below). The use of CBCT as a tool for trabecular pattern description lies mainly in presurgical bone quality assessment and in cases where clinical complaints cannot be resolved using two-dimensional imaging. We are currently conducting studies on the diagnostic performance of CBCT and its value in therapeutical planning and outcome.

## FACTORS INFLUENCING JAW BONE QUALITY

In Chapter 5, we investigated the diagnostic accuracy of jaw bone density on intra-oral radiographs for osteoporosis detection. There was indeed a dissimilar distribution of the density between individuals with and without osteoporosis, but also a considerable overlap. To provide a more powerful screening tool it is advisable to include other parameters in the assessment. As a successful example of multi-parameter approach in osteoporosis screening, Devlin et al (2008) used a combination of panoramic mandibular width and the OSIRIS index, including age, weight, hormone replacement therapy and history of low trauma fractures and found an area under the ROC curve of 0.90 for detecting osteoporosis, exceeding the diagnostic performance of the mandibular width and OSIRIS applied separately.

The importance of several clinical data when assessing jaw bone became evident from previous research. Age and gender data are easy to collect and of high relevance in bone characteristics. In older individuals, the mandible is characterized by having a relatively thin porous cortex showing erosion in the endosteal area and a decrease in bone mineral density (BMD) (Gulsahi et al 2008, Devlin et al 2007b). The age related cortical bone loss as well as the BMD loss are more pronounced in females than in males (von Wowern 1986). Using intra-oral radiographs for assessing mandibular bone, Jonasson et al 2007 concluded that alveolar bone thickness and bone density are larger, and bone structure denser, for men than for women.

After tooth loss, the mandible shows continuous alveolar ridge atrophy. However, this height reduction does not seem to be accompanied by a reduction in bone quality. Devlin et al (2007b) could not identify dental state as a significant explanatory factor in the prediction of mandibular bone mineral density. Other authors confirm this finding (Celenk & Celenk 2008, Choel et al 2004). There is a large interindividual difference in the rate of residual ridge reduction, for which the cause is until now not well understood. Apparently, too many possible factors can account for the rate, not one being the dominant factor (Carlsson 2004). To avoid jaw bone resorption preventive dental care needs to be optimal, to let people keep their natural teeth as long as possible. In edentulous jaws, implant-supported prostheses can reduce bone loss or even promote bone growth (Carlsson 2004).

When evaluating bone density or structure, and especially using this information in comparative research, the selection of the location is of utmost importance. Because of functional loading during mastication, the interdental crestal region has more dense trabeculation than the apical region (Jonasson et al 2007). Park et al (2008) performed an extensive study on the density of the jaw bones using CT and found the highest and lowest maxillary bone density respectively in the canine and premolar areas and the maxillary tuberosity. Mandibular cortical bone was denser in the mandible than in the maxilla and increased from the incisor to the retromolar area. Cortical bone was denser when measured at the basal area, compared to the alveolar ridge. For trabecular bone, this was vice versa: it was denser at the alveolar ridge than at the basal area. When assessing density it is important not to include parts of teeth in the region of

interest, since these would increase the density. This hinders the selection of a region, which forms a lesser problem when structural properties of bone are the object of study (Geraets et al 2008).

Besides age, gender, dental state and location, other systemic and local factors can influence the outcome of bone quality assessment. These include periodontal or endodontic bone lesions, extractions or other local bone pathology, drug therapy, life style factors (Chapter 6) and systemic disease, such as osteoporosis (cfr below).

## OSTEOPOROSIS SCREENING

Osteoporosis is a prevalent disease and causes a burden for the individual as well as the socioeconomic system. For physicians, it is a challenge to identify individuals at high risk of fracture. It might be possible for general dentists to be involved in this challenge, as was expressed in the clinical study presented in this thesis. Besides the method of jaw bone density assessment on intra-oral radiographs, other approaches are taken for screening purposes.

Various studies performed under the European Osteodent project have shown the use of several methods in oral radiology for osteoporosis screening (Karayianni et al 2006, Geraets et al 2007, Devlin et al 2007a & c, Horner et al 2007, Lindh et al 2008, Nackaerts et al 2008). All techniques used, confirm that, jaw bone quality is indicative of systemic bone density. Geraets et al (2007) performed fractal analysis of the trabecular bone structure on intra-oral radiographs and panoramic radiographs and found that, in the prediction of BMD, this quantitative analysis is feasible, with an area under the ROC curve comparable to that of commonly used screening instruments for osteoporosis. Lindh et al (2008) used visual assessment of intra-oral radiographs for identifying women at risk of having osteoporosis and found high specificity for this method but sensitivity was low. Moreover, the problem of interobserver differences cannot be disregarded in subjective assessment methods. The mandibular cortical index (MCI), another subjective method discussed before, showed rather limited value for osteoporosis screening when compared to the clinical risk index OSIRIS (Horner et al 2007). Also when compared to the objective measure of mandibular cortical width, MCI had a lower predictive value for osteoporosis detection (Devlin

et al 2007c). Including OSIRIS in manual cortical width measurements improved specificity, though at the expence of sensitivity (Karayianni et al 2006). Further research should therefore address the cost/benefit issue, to create a test compatible to local healthcare facilities. Devlin et al (2007a) made further steps towards the elimination of observer influence in their measurement of cortical width. Their group developed software to automatically detect the cortical border and found promising results for what concerns diagnostic ability and repeatability. In combination with the OSIRIS index, this automated cortical width measurement (Active Shape Model search, Cootes et al 1995) reached an area under the ROC curve of 0.90 and as such proved to be a suitable method for osteoporosis screening in oral health care (Devlin et al 2008).

## OSTEOPOROSIS DIAGNOSIS

The use of a diagnostic test lies in detecting people who have a disease and in showing a negative result in people that do not have the disease. To obtain this ability, test results are compared to a gold standard, the 'true status' of the patient. In the Osteodent project this gold standard was BMD of the hip and spine as measured by DXA, being the gold standard for diagnosing osteoporosis.

Indeed, hip fracture risk increases as hip BMD decreases, but some reflections should be made. First, BMD measured by DXA has high specificity but lacks sensitivity, losing a number of high-risk individuals at a moment where preventive action would still be possible (World Health Organisation 1994). Second, epidemiological studies have shown that a substantial proportion of osteoporotic fractures occur in women who do not meet BMD criteria for osteoporosis (Schuit et al 2004). Bone density is not the sole determinant of fracture risk. Neuromuscular function and environmental factors influence the propensity to fall. The geometry, microarchitecture and quality of bone are all components determining bone strength and therefore the occurrence of low trauma fractures. Therefore, researchers are aiming towards a combined approach for assessing fracture risk. The use of BMD keeps its merits, but should be complemented with validated clinical risk factors (Fardellone 2008) and/or bone architectural indices (Apostol et al 2006). In the meantime, WHO has created FRAX (WHO Fracture Risk Assessment Tool www.shef.ac.uk/FRAX). It is based on individual patient models

that integrate the risks associated with clinical risk factors as well as bone mineral density at the femoral neck. The output of the FRAX algorithms is a 10-year probability of hip fracture and the 10-year probability of a major osteoporotic fracture (clinical spine, forearm, hip or shoulder fracture). This message of the importance of fracture risk assessment should be transferred to research, focusing on osteoporosis screening in oral health care. It is advisable to perform longitudinal studies and focus on fracture risk assessment, rather than the prediction of BMD alone. White et al (2005) performed such a study and found that the rate of hip fracture was correlated with trabecular architecture. Inclusion of clinical parameters improved the predictive model substantially, a confirmation of the importance of including clinical parameters within models for osteoporosis screening.

## CONCLUSIONS

The ultimate goal of this thesis was to establish a strategy for bone density assement on routine intra-oral radiographs. While the first study basically illustrated the shortcomings of panoramic imaging with regard to bone density (Chapter 2), the subsequent papers developed, validated and used the technique for density assessment in intra-oral radiographs (Chapter 3, 4) using a reference wedge in animal research (Chapter 7) and under clinical conditions (Chapter 5 & 6). The clinical studies proved the applicability of the technique, but if clinical factors as age, BMI … could be added it might yield more convincing sensitivity/specificity for osteoporosis detection. We linked jaw bone density data to other factors influencing jaw bone quality such as alcohol consumption. Finally the inherent shortcomings of 2D images were overcome when CBCT came into play (Chapter 8). CBCT, although not suitable in a screening context, is a promising tool for bone evaluation. We will continue to study its use in a diagnostic and therapeutical context.

# Summary

# Samenvatting

## SUMMARY

The assessment of jaw bone quality has a broad field of potential applications, such as preoperative planning of implant placement or the follow-up of bone changes as a result of progression of disease or therapy. Clinical tools for objective quantification of jaw bone quality are sparse, and often not validated. Therefore, the overall aim of this thesis was to develop and validate an objective, clinically applicable tool for jaw bone quality evaluation.

Initially, panoramic radiographs were used for assessing jaw bone quality and its predictive value for the degree of marginal bone loss (Chapter 2). Bone quality was assessed by radiographic density and a bone quality index. Although radiographic density of alveolar bone was associated with periodontal bone level to some extent, we were confronted with some methodological constraints of the use of panoramic radiographs, such as geometrical distortion and spatial resolution.

The search for a bone density evaluation tool was continued using intra-oral radiographs. For objective quantification, an aluminium stepwedge was chosen as a reference on the radiographs. This had practical consequences for the planned clinical studies which is why, in Chapter 3, the benefit of using such a stepwedge was explored and confirmed. Densitometric assessment showed good reproducibility for the analyses with and without aluminium wedge correction, but the assessment was far more accurate when the wedge was included.

After this initial justification, the tool was further developed and validated in Chapter 4. Accuracy and precision were determined in an *in vitro* study. The minimal detection threshold was also determined. The tool showed potential for bone density evaluation to monitor minute bone changes and was subsequently applied in an *in vivo* study.

Chapter 5 describes this clinical study, which was set up to identify persons with osteoporosis using the tool for jaw bone densitometry on intra-oral radiographs. A fair diagnostic accuracy for osteoporosis in the hip or spine was obtained. Based on the same population, in Chapter 6, the influence of one specific lifestyle factor on the jaw bone, was explored: alcohol consumption. We found a reduced density

and mandibular cortical thickness in persons with increased alcohol consumption, as well as in older individuals.

For another *in vivo* application of the tool (Chapter 7), we evaluated the impact of replacement therapy by monitoring bone density changes and alveolar bone level in periodontal pockets in a dog model. Although in a small sample, we could appreciate the value of the densitometric tool as a follow-up instrument for detecting small changes in bone density.

The densitometric tool for jaw bone has proven its use in the objective assessment of bone. It must be added that it might be necessary to obtain more specific information on the structural properties of bone to correctly interpret certain clinical situations. With a look into the future, we performed a study in Chapter 8 to obtain an idea on how the density on radiographs was correlated to the bone structure on microscopy. The density derived from periapical radiographs showed good correlation with bone structural characteristics, such as bone area and cortical width.

## SAMENVATTING

Het beoordelen van de kwaliteit van kaakbot heeft een breed toepassingsveld, bijvoorbeeld bij preoperatieve planning van implantaten of het opvolgen van botveranderingen onder invloed van ziekteprocessen of therapeutische handelingen. Klinisch bruikbare methodes voor het objectief kwantificeren van kaakbotkwaliteit zijn eerder schaars en vaak niet gevalideerd. Het hoofddoel van deze thesis was dan ook het ontwikkelen en valideren van een objectieve, klinisch bruikbare methode voor het evalueren van botkwaliteit.

Aanvankelijk werden panoramische radiografieën gebruikt voor het beoordelen van kaakbotkwaliteit en de mogelijkheid om met deze informatie de mate van marginaal botverlies te voorspellen (Hoofdstuk 2). Botkwaliteit werd beoordeeld op basis van grijswaarden en met behulp van een botkwaliteitsindex. De gevonden densiteit bleek in zekere mate het alveolaire botniveau te beïnvloeden. Toch werden we met de methodologische beperkingen van panoramische radiografieën geconfronteerd, zoals de geometrische vervorming en spatiale resolutie.

De methode voor het evalueren van botkwaliteit werd vervolgens gezocht bij intra-orale radiografieën. Om een objectieve waarde te bekomen werd een aluminium wig als referentie op de beeldreceptor geplaatst. Dit had praktische gevolgen voor de geplande klinische studies. Daarom werd in Hoofdstuk 3 bekeken of en bevestigd dat dergelijke wig werkelijk een meerwaarde bood. Densiteitsbepalingen waren reproduceerbaar, zowel met als zonder de wig, maar de resultaten met de wig waren veel accurater voor het inschatten van de werkelijke densiteit.

Na deze eerste exploratie werd de methode verder ontwikkeld en gevalideerd in Hoofdstuk 4. Accuraatheid en precisie werden nagegaan bij een *in vitro* studie set-up. Daarnaast werd de detectiedrempel voor botveranderingen bepaald. De methode bewees bruikbaar te zijn voor het meten van botdensiteit en het opvolgen van kleine veranderingen daarin en werd vervolgens in een *in vivo* studie toegepast.

Hoofdstuk 5 beschrijft deze klinische studie, die werd opgestart met als doel het identificeren van personen met osteoporose met behulp van kaakbotdensiteitsmetingen. De diagnostische waarde van de densiteitsmetingen op kaakbot was goed. Gebaseerd op dezelfde populatie werd in Hoofdstuk 6 de

invloed van alcohol consumptie op kaakbot onderzocht. We vonden een lagere densiteit en een lagere mandibulaire corticale dikte bij personen met een hoge alcoholconsumptie, en bij oudere personen.

Een volgende toepassing bestond erin de impact van therapie voor parodontitis te evalueren door het opvolgen van botdensiteit (Hoofdstuk 7). Het betrof een studie naar het effect van beneficiële microbiota in een hondenmodel. Hoewel de studiepopulatie bescheiden was, werd de waarde van de methode voor het opvolgen van kleine botdensiteitsveranderingen duidelijk.

De ontwikkelde methode bewees haar nut voor het objectief beoordelen van kaakbot. Hierbij dient opgemerkt dat soms ook structurele karakteristieken van het bot nodig zijn voor het correct inschatten van klinische situaties. Met het oog op de toekomst voerden we in Hoofdstuk 8 een validatiestudie uit om na te gaan hoe de densiteit op intra-orale radiografieën gecorreleerd was met de botstructuur op microscopische beelden. De densiteit toonde een hoge correlatie met structurele eigenschappen zoals de relatieve botoppervlakte en de corticale breedte.

# References

Allen PD, Graham J, Farnell DJ, Harrison EJ, Jacobs R, Karayianni K, Lindh C, van der Stelt PF, Horner K, Devlin H. Detecting reduced bone mineral density from dental radiographs using statistical shape models. IEEE Trans Inf Technol Biomed 2007;11:601-610

Apostol L, Boudousq V, Basset O, Odet C, Yot S, Tabary J, Dinten JM, Boller E, Kotzki PO, Peyrin F. Relevance of 2D radiographic texture analysis for the assessment of 3D bone micro-architecture. Med Phys 2006;33:3546-3556

Aranyarachkul P, Caruso J, Gantes B, Schulz E, Riggs M, Dus I, Yamada JM, Crigger M. Bone density assessments of dental implant sites: 2. Quantitative cone-beam computerized tomography. Int J Oral Maxillofac Implants 2005;20:416–424

Bahrami G, Wenzel A, Kirkevang LL, Isidor F, Vaeth M. Risk indicators for a reduced marginal bone level in the individual. Oral Health Prev Dent 2006;4:215-222

Bainbridge KE, Sowers MF, Crutchfield M, Lin X, Jannausch M, Harlow SD. Natural history of bone loss over 6 years among premenopausal and early postmenopausal women. Am J Epidemiol 2002;156:410-417

Benson B, Prihoda J, Glass B. Variations in adult cortical bone mass as measured by a panoramic mandibular index. Oral Surg Oral Med Oral Pathol 1991;71:349-356

Berkhout WE, Sanderink GC, Van der Stelt PF. A comparison of digital and film radiography in Dutch dental practices assessed by questionnaire. Dentomaxillofac Radiol 2002;31:93-99

Bohn MJ, Babor TF, Kranzler HR. The Alcohol Use Disorders Identification Test (AUDIT): validation of a screening instrument for use in medical settings. J Stud Alcohol 1995;56:423-432

Bonnick SL. Densitometry techniques. In Bonnic SL (ed), Bone densitometry in clinical practice (pp 1–24). 2004, Totowa, NJ: Humana Press

Borg E, Grondahl K, Persson LG, Grondahl HG. Marginal bone level around implants assessed in digital and film radiographs: *in vivo* study in the dog. Clin Impl Dent Rel Res 2000;2:10-17

Brägger U, Pasquali L, Rylander H, Carnes D, Kornman KS. Computer-assisted densitometric image analysis in periodontal radiography. J Clin Periodontol 1988;15:27-37

Brägger U. Radiographic parameters: biological significance and clinical use. Periodontology 2000 2005;39:73-90

Brot C, Jorgensen NR, Sorensen OH. The influence of smoking on vitamin D status and calcium metabolism. Eur J Clin Nutr 1999;53:920-926

Bryant SR. The effects of age, jaw site, and bone condition on oral implant outcomes. Int J Prosthodont 1998;11:470-490

Cadarette SM, Jaglal SB, Kreiger N, McIsaac WJ, Darlington GA, Tu JV. Development and validation of the Osteoporosis Risk Assessment Instrument to facilitate selection of women for bone densitometry. CMAJ 2000;162:1289–1294

Callaci JJ, Juknelis D, Patwardhan A, Wezeman FH. Binge alcohol treatment increases vertebral bone loss following ovariectomy: compensation by intermittent parathyroid hormone. Alcohol Clin Exp Res 2006;30:665-672

Carlsson GE. Responses of jawbone to pressure. Gerodontol 2004;21:65-70

Celenk C, Celenk P. Evaluation by quantitative magnetic resonance imaging of trabecular bone quality in the dentate and edentulous mandible. Clin Oral Implants Res 2008;19:15-18

Choel L, Last D, Duboeuf F, Seurin MJ, Lissac M, Briguet A, Guillot G. Trabecular alveolar bone microarchitecture in the human mandible using high resolution magnetic resonance imaging. Dentomaxillofac Radiol 2004;33:177–182

Christgau M, Hiller KA, Schmalz G, Kolbeck C, Wenzel A. Accuracy of quantitative digital subtraction radiography for determining changes in calcium mass in mandibular bone: an *in vitro* study. J Period Res 1998;33:138-149

Civitelli R, Pilgram TK, Dotson M, Muckerman J, Lewandowski N, Armamento-Villareal R, Yokoyama-Crothers N, Kardaris EE, Hauser J, Cohen S, Hildebolt CF. Alveolar and postcranial bone density in postmenopausal women receiving hormone/estrogen replacement therapy: a randomized, double-blind, placebo-controlled trial. Arch Intern Med 2002;24:1409–1415

Consensus development conference. Prophylaxis and treatment of osteoporosis. Am J Med 1991;90:107–110

Cornuz J, Feskanich D, Willett WC, Colditz GA. Smoking, smoking cessation, and risk of hip fracture in women. Am J Med 1999;106: 311-314

Corten FG, Van ‘t Hof MA, Buijs WC, Hoppenbrouwers P, Kalk W, Corstens FH. Measurement of mandibular bone density *ex vivo* and *in vivo* by dual-energy X-ray absorptiometry. Arch Oral Biol 1993;38:215–219

Couto Souza P, Pereira da Costa N, Bauer Veeck E. Influence of soft tissues on mandibular gray scale levels. Braz Oral Res 2004;18:40-44

Dawson DA. Consumption indicators of alcohol dependence. Addiction 1994;89:345-350

Dervis E. Oral implications of osteoporosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endo 2005;100:349–356

Devlin H[a], Allen PD, Graham J, Jacobs R, Karayianni K, Lindh C, van der Stelt P, Harrison E, Adams JE, Pavitt S, Horner K. Automated osteoporosis risk assessment by dentists: A new pathway to diagnosis. Bone 2007;40:835–842

Devlin H, Allen PD, Graham J, Jacobs R, Karayianni K, Lindh C, van der Stelt PF, Marjanovic E, Adams JE, Pavitt S, Horner K. The Role of the Dental Surgeon in Detecting Osteoporosis: the OSTEODENT Study. Br Dent J 2008;204:E16

Devlin H, Ferguson MWJ. Alveolar ridge resorption and mandibular atrophy. A review of the role of local and systemic factors. Br Dent J 1991;170:101-104

Devlin H, Horner K, Ledgerton D. A comparison of maxillary and mandibular bone mineral densities. J Prosthet Dent 1998;79:323–327

Devlin H[b], Horner K. A study to assess the relative influence of age and edentulousness upon mandibular bone mineral density in female subjects. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:117-121

Devlin H, Horner K. Mandibular radiomorphometric indices in the diagnosis of reduced skeletal bone mineral density. Osteoporosis Int 2002;13:373–378

Devlin H[c], Karayianni K, Mitsea A, Jacobs R, Lindh C, van der Stelt P, Marjanovic E, Adams J, Pavitt S, Horner K. Diagnosing osteoporosis by using dental panoramic radiographs: The OSTEODENT project. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:821–828

Dey P. Basic principles and applications of fractal geometry in pathology: a review. Anal Quant Cytol Histol 2005;27:284-290

Diamond T, Stiel D, Lunzer M, Wilkinson M, Posen S. Ethanol reduces bone formation and may cause osteoporosis. Am J Med 1989;86:282-288

Dornier C, Dorsaz-Brossa L, Thevenaz P, Casagni F, Brochut P, Mombelli A, Vallee J. Geometric alignment and chromatic calibration of serial radiographic images. Dentomaxillofac Radiol 2004;33:220–225

Drage NA, Palmer RM, Blake G, Wilson R, Crane F, Fogelman I. A comparison of bone mineral density in the spine, hip and jaws of edentulous subjects. Clin Oral Implants Res 2007;18:469-500

Dreyer WP. Technological advances in the clinical diagnosis of periodontal diseases. Int Dent J 1993;43:557–566

Drozdzowska B, Pluskiewicz W, Tarnawska B. Panoramic-based mandibular indices in relation to mandibular bone mineral density and skeletal status assessed by dual energy X-ray absorptiometry and quantitative ultrasound. Dentomaxillofac Radiol 2002;31:361–367

Du Tré F, Jacobs R, Styven S, van Steenberghe D. Development of a novel digital subtraction technique for detecting subtle changes in jawbone density. Clin Oral Investig 2006;10:235–248

Dural S, Ozbek M, Kanli A, Orhan K, Kanbur NO, Derman O, Delilbasi C. Evaluation of mandibular bone density to predict osteoporosis in adolescents with constitutional delayed growth. Saudi Med J 2005;26:1235–1239

Eickholz P, Horr T, Klein F, Hassfeld S, Kim TS. Radiographic parameters for prognosis of periodontal healing of infrabony defects: two different definitions of defect depth. J Periodontol 2004;75:399-407

Eickholz P, Krigar DM, Kim TS, Reitmeir P, Rawlinson A. Stability of clinical and radiographic results after guided tissue regeneration in infrabony defects. J Periodontol 2007;78:37-46

Elders PJ, Habets LL, Netelenbos JC, Linden LW, Stelt PF. The relation between periodontitis and systemic bone mass in women between 46 and 55 years of age. J Clin Periodontol 1992;19:492–496

Elders PJ, Netelenbos JC, Lips P, van Ginkel FC, van der Stelt PF. Accelerated vertebral bone loss in relation to the menopause: a cross-sectional study on lumbar bone density in 286 women of 46 to 55 years of age. Bone and Mineral 1988;5:15-17

Faber TD, Yoon DC, Service SK, White SC. Fourier and wavelet analyses of dental radiographs detect trabecular changes in osteoporosis. Bone 2004;35:403–411

Famili P, Cauley J, Suzuki JB, Weyant R. Longitudinal study of periodontal disease and edentulism with rates of bone loss in older women. J Periodontol 2005;76:11–15

Fardellone P. Predicting the fracture risk in 2008. Joint Bone Spine 2008;75:661-664

Felson DT, Zhang Y, Hannan MT, Kannel WB, Kiel DP. Alcohol intake and bone mineral density in elderly men and women. The Framingham Study. Am J Epidemiol 1995;142:485-492

Fleming MF, Mundt MP, French MT, Manwell LB, Stauffacher EA, Barry KL. Brief physician advice for problem drinkers: long-term efficacy and benefit-cost analysis. Alcohol Clin Exp Res 2002;26:36-43

Fonseca AA, Cherubini K, Veeck EB, Ladeira RS, Carapeto LP. Effect of 10% formalin on radiographic optical density of bone specimens. Dentomaxillofac Radiol 2008;37:137-141

Friedlander LT, Love RM, Chandler NP. A comparison of phosphor-plate digital images with conventional radiographs for the perceived clarity of fine endodontic files and periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endo 2002;93:321–327

Geraets WG, van der Stelt P. Fractal properties of bone. Dentomaxillofac Radiol 2000;29:144-153

Geraets WG, van der Stelt PF, Elders PJM. The radiographic trabecular bone pattern during menopause. Bone 1993;14:859–864

Geraets WG, van der Stelt PF, Lips P, van Ginkel FC. The radiographic trabecular pattern of hips in patients with hip fractures and in elderly control subjects. Bone 1998;22:165–173

Geraets WG, van der Stelt PF. Analysis of the radiographic trabecular pattern. Pattern Recognition Letters 1991;12:575–581

Geraets WG, van Ruijven LJ, Verheij JG, van Eijden TM, van der Stelt PF. A sensitive method for measuring spatial orientation in bone structures. Dentomaxillofac Radiol 2006;35:319–325

Geraets WG, Verheij JG, van der Stelt PF, Horner K, Lindh C, Nicopoulou-Karayianni K, Jacobs R, Harrison EJ, Adams JE, Devlin H. Prediction of bone mineral density with dental radiographs. Bone 2007;40:1217-1221

Geraets WG, Verheij JGC, van der Stelt PF, Horner K, Lindh C, Nicopoulou-Karayianni K, Jacobs R, Marjanovic EJ, Adams JE, Devlin H. Selecting regions of interest on intra-oral radiographs for the prediction of bone mineral density. Dentomaxillofac Radiol 2008;37:375-379

Geurs NC, Lewis CE, Jeffcoat MK. Osteoporosis and periodontal disease progression. Periodontol 2000 2003;32:105–110

Geusens P, Hochberg MC, van der Voort DJ, Pols H, van der Klift M, Siris E, Melton ME, Turpin J, Byrnes C, Ross P. Performance of risk indices for identifying low bone density in postmenopausal women. Mayo Clin Proc 2002;77:629–637

Gijbels F, Debaveye D, Vanderstappen M, Jacobs R. Digital radiographic equipment in the Belgian dental office. Radiat Prot Dosimetry 2005;117:309-312

Gomes-Filho IS, Passos JS, Cruz SS, Vianna MI, Cerqueira EM, Oliveira DC, dos Santos CA, Coelho JM, Sampaio FP, Freitas CO, de Oliveira NF. The association between postmenopausal osteoporosis and periodontal disease. J Periodontol 2007;78:1731-1740

Goodson JM, Haffajee AD, Socransky SS. The relationship between attachment level loss and alveolar bone loss. J Clin Periodontol 1984;11:348–359

Guerrero ME, Jacobs R, Loubele M, Schutyser F, Suetens P, van Steenberghe D. State-of-the-art on CBCT imaging for preoperative planning of implant placement. Clin Oral Investig 2006;10:1-7

Gulsahi A, Yüzügüllü B, Imirzalioglu P, Genç Y. Assessment of panoramic radiomorphometric indices in Turkish patients of different age groups, gender and dental status. Dentomaxillofac Radiol 2008;37:288-292

Haentjens P, Autier P, Collins J, Valkeniers B, Vanderschueren D, Boonen S. Colles fracture, spine fracture and subsequent risk of hip fracture in men and women. A meta-analysis. J Bone Joint Surg Am 2003;85:1936–1943

Haffar IA, Padilla F, Nefussi R, Kolta S, Foucart J-M, Laugier P. Experimental evaluation of bone quality measuring speed of sound in cadaver mandibles. Oral Surg Oral Med Oral Pathol Oral Radiol Endo 2006;102:782-791

Hangiandreou NJ, O'Connor TJ, Felmlee TP. An evaluation of the signal and noise characteristics of four CCD-based film digitizers. Med Phys 1998;25:2020-2026

Hausmann E, Allen K, Clerehugh V. What alveolar crest level on a bite-wing radiograph represents bone loss. J Periodontol 1991;62:570–572

Hausmann E. Radiographic and digital imaging in periodontal practice. J Periodontol 2000;71:497-503

Hermann I, Lekholm U, Holm S, Kultje C. Evaluation of patient and implant characteristics as potential prognostic factors for oral implant failures. Int J Oral Maxillofac Implants 2005;20:220-230

Herod EL. The effect of cheese on dental caries: a review of the literature. Austr Dent J 1991;36:120-125

Hildebolt CF, Pilgram TK, Yokoyama-Crothers N, Vannier MW, Dotson M, Muckerman J, Armamento-Villareal R, Hauser J, Cohen S, Kardaris EE, Hanes P, Shrout MK, Civitelli R. The pattern of alveolar crest height change in healthy postmenopausal women after 3 years of hormone/estrogen replacement therapy. J Periodontol 2002;73:1279–1284

Holbrook TL, Barrett-Connor E. A prospective study of alcohol consumption and bone mineral density. Br Med J 1993;306:1506-1509

Holland M, Boring JG, Boyle CR, Pickrum HM, Jeffcoat MK. Radiographic bone loss correlations and technetium-99m-MDP bone uptake in ligature-induced periodontal disease in the beagle. Veterinary Radiology and Ultrasound 1998;39:366-374

Horner K, Devlin H. Clinical bone densitometric study of mandibular atrophy using dental panoramic tomography. J Dent 1992;20:33–37

Horner K, Devlin H, Alsop CW, Hodgkinson IM, Adams JE. Mandibular bone mineral density as a predictor of skeletal osteoporosis. Br J Radiol 1996;69:1019–1025

Horner K, Devlin H, Harvey L. Detecting patients with low skeletal bone mass. J Dent 2002;30:171–175

Horner K, Devlin H. The relationship between mandibular bone mineral density and panoramic radiographic measurements. J Dent 1998;26:337–343

Horner K, Karayianni K, Mitsea A, Berkas L, Mastoris M, Jacobs R, Lindh C, van der Stelt P, Marjanovic E, Adams J, Pavitt S, Devlin H. The mandibular cortex on radiographs as a tool for osteoporosis risk assessment: The OSTEODENT project. J Clin Densitom 2007;10:138–146

Hujoel PP, DeRouen TA. Validity issues in split-mouth trials. J Clin Periodont 1992;19:625-627

Huumonen S, Kvist T, Gröndahl K, Molander A. Diagnostic value of computed tomography in re-treatment of root fillings in maxillary molars. Int Endod J 2006;39:827-833

Inagaki K, Kurosu Y, Yoshinari N, Noguchi T, Krall EA, Garcia RI. Efficacy of periodontal disease and tooth loss to screen for low bone mineral density in Japanese women. Calcif Tissue Int 2005;77:9-14

Iqbal MK, Kim S. A review of factors influencing treatment planning decisions of single-tooth implants versus preserving natural teeth with nonsurgical endodontic therapy. J Endod 2008;34:519-529

Jacobs R, Adriansens A, Verstreken K, Suetens P, van Steenberghe D. Predictability of a three-dimensional planning system for oral implant surgery. Dentomaxillofac Radiol 1999;28:105-111

Jacobs R, van Steenberghe D. Radiographic planning and assessment of endosseous oral implants. 1998, Springer, Berlin

Jacobs R, Ghyselen J, Koninckx P, van Steenberghe D. Long-term bone mass evaluation of mandible and lumbar spine in a group of women receiving hormone replacement therapy. Eur J Oral Sci 1996;104:10–16

Jemt T, Lekholm U. Implant treatment in edentulous maxillae: A 5-year prospective multicenter follow-up report on patients with different degrees of jaw resorption. Int J Oral Maxillofac Implants 1995;10:303-311

Jonasson G. Mandibular alveolar bone mass, structure and thickness in relation to skeletal bone density in dentate women. Swed Dent J Suppl 2005;177:1–63

Jonasson G, Bankvall G, Kiliaridis S. Estimation of skeletal bone mineral density by means of the trabecular pattern of the alveolar bone, its interdental

thickness, and the bone mass of the mandible. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92:346–352

Jonasson G, Kiliaridis S. Changes in the bucco-lingual thickness of the mandibular alveolar process and skeletal bone mineral density in dentate women: a 5-yr prospective study. Eur J Oral Sci 2005;113:114–120

Kanis JA, Johansson H, Johnell O, Oden A, De Laet C, Eisman JA, Pols H, Tenenhouse A. Alcohol intake as a risk factor for fracture. Osteoporosis Int 2005;16:737-742

Karayianni K, Horner K, Mitsea A, Berkas L, Mastoris M, Jacobs R, Lindh C, van der Stelt PF, Harrison E, Adams JE, Pavitt S, Devlin H. Accuracy in osteoporosis diagnosis of a combination of mandibular cortical width measurement on dental panoramic radiographs and a clinical risk index (OSIRIS): The OSTEODENT project. Bone 2007;40:223–229

Katsumata A, Hirukawa A, Okumura S, Naitoh M, Fujishita M, Ariji E, Langlais RP. Effects of image artefacts on gray-value density in limited-volume cone-beam computerized tomography. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:829-836

Klemetti E, Kolmakov S, Heiskanen P, Vainio P, Lassila V. Panoramic mandibular index and bone mineral densities in postmenopausal women. Oral Surg Oral Med Oral Pathol 1993;75:774–779

Klemetti E, Kolmakov S, Kroger H. Pantomography in assessment of the osteoporosis risk group. Scand J Dent Res 1994;102:68–72

Klemetti E, Kolmakow S. Morphology of the mandibular cortex on panoramic radiographs as an indicator of bone quality. Dentomaxillofac Radiol 1997;26:22–25

Knezovic-Ziataric D, Celebic A. Mandibular bone mineral density changes in complete and removable partial denture wearers: a 6-month follow-up study. Int J Prosthodont 2003;16:661–665

Kribbs PJ. Comparison of mandibular bone in normal and osteoporotic women. J Prosthet Dent 1990;63:218–222

Landini G. Is periodontal breakdown a fractal process? Simulations using the Weierstrass–Mandelbrot function. J Periodontal Res 1997;32:300–307

Law AN, Bollen AM, Chen SK. Detecting osteoporosis using dental radiographs: a comparison of four methods. J Am Dent Assoc 1996;127:1734–1742

Ledgerton D, Horner K, Devlin H, Worthington H. Panoramic mandibular index as a radiomorphometric tool: an assessment of precision. Dentomaxillofac Radiol 1997;26:95-100

Lee BD, White SC. Age and trabecular features of alveolar bone associated with osteoporosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:92–98

Lee K, Taguchi A, Ishii K, Suei Y, Fujita M, Nakamoto T, Ohtsuka M, Sanada M, Tsuda M, Ohama K, Tanimoto K, White SC. Visual assessment of the mandibular cortex on panoramic radiographs to identify postmenopausal women with low bone mineral densities. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:226–231

Lehmann TM, Troeltsch E, Spitzer K. Image processing and enhancement provided by commercial dental software programs. Dentomaxillofac Radiol 2002;31:264–272

Lekholm U, Zarb GA. Patient selection and preparation. In Brånemark P-I, Zarb GA, Albrektsson T (eds.), Tissue-integrated prostheses. Osseointegration in clinical dentistry (pp 199–209). 1985, Quintessence, Chicago

Lerner UH. Inflammation-induced bone remodelling in periodontal disease and the influence of post-menopausal osteoporosis. J Dent Res 2006;85:596-607

Li G, Engstrom PE, Nasstrom K, Lu ZY, Sanderink G, Welander U. Marginal bone levels measured in film and digital radiographs corrected for attenuation and visual response: an *in vivo* study. Dentomaxillofac Radiol 2007;36:7-11

Lindh C, Petersson A, Rohlin M. Assessment of the trabecular pattern before endosseous implant treatment: diagnostic outcome of periapical radiography in the mandible. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82:335–343

Looker A, Wahner H, Dunn W, Calvo M, Harris T, Heyse S, Johnston CC Jr, Lindsay R. Updated data on proximal femur bone mineral levels of US adults. Osteoporos Int 1998;8:468–489

Loubele M, Bogaerts R, Van Dijck E, Pauwels R, Vanheusden S, Suetens P, Marchal G, Sanderink G, Jacobs R. Comparison between effective radiation

dose of CBCT and MSCT scanners for dentomaxillofacial applications. Eur J Radiol 2008;e-pub

Lundström A, Jendle J, Stenström B, Toss G, Ravald N. Periodontal conditions in 70-year-old women with osteoporosis. Swed Dent J 2001;25:89–96

Mandelbrot BB. The fractal geometry of nature. 3rd ed. 1983, New York, WH Freeman & Co

Manolagas SC, Jilka RL. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med 1995;332:305-311

Matteson SR, Deahl ST, Alder ME, Nummikoski PV. Advanced imaging methods. Crit Rev Oral Biol Med 1996;7:346-395

Mauck KF, Cuddihy M-T, Atkinson EJ, Melton J. Use of clinical prediction rules in detecting osteoporosis in a population-based sample of postmenopausal women. Arch Intern Med 2005;165:530–536

McCaul LK, McHugh S, Saunders WP. The influence of specialty training and experience on decision making in endodontic diagnosis and treatment planning. Int Endod J 2001;34:594–606

Melton LJ 3rd, Kanis JA, Johnell O. Potential impact of osteoporosis treatment on hip fracture trends. J Bone Miner Res 2005;20:895–897

Mohajery M, Brooks SL, Detroit, Arbor A. Oral radiographs in the detection of early signs of osteoporosis. Oral Surg Oral Med Oral Pathol 1992;72:112–117

Mohammad AR, Hooper DA, Vermilyea SG, Mariotti A, Preshaw PM. An investigation of the relationship between systemic bone density and clinical periodontal status in post-menopausal Asian-American women. Int Dent J 2003;53:121–125

Mukamal KJ, Robbins JA, Cauley JA, Kern LM, Siscovick DS. Alcohol consumption, bone density, and hip fracture among older adults: the cardiovascular health study. Osteoporosis Int 2007;18:593-602

Müller H-P, Ulbrich M. Alveolar bone levels in adults as assessed on panoramic radiographs. (I) Prevalence, extent, and severity of even and angular bone loss. Clin Oral Invest 2005;9:98–104

Müller H-P, Ulbrich M, Heinecke A. Alveolar bone loss in adults as assessed on panoramic radiographs. (II) Multilevel models. Clin Oral Invest 2005;9:105–110

Nackaerts O, Jacobs R, Devlin H, Pavitt S, Bleyen E, Yan B, Borghs H, Lindh C, Karayianni K, van der Stelt P, Marjanovic E, Adams JE, Horner K. Osteoporosis detection using intra-oral densitometry. Dentomaxillofac Radiol 2008;37:282-287

Nackaerts O, Jacobs R, Horner K, Zhao F, Lindh C, Karayianni K, van der Stelt P, Pavitt S, Devlin H. Bone density measurements in intra-oral radiographs. Clin Oral Investig 2007;11:225-229

Nackaerts O, Jacobs R, Pillen M, Engelen L, Gijbels F, Devlin H, Lindh C, Nicopoulou-Karayianni K, van der Stelt P, Pavitt S, Horner K. Accuracy and precision of a densitometric tool for jaw bone. Dentomaxillofac Radiol 2006;35:244-248

Nakamoto T, Taguchi A, Ohtsuka M, Suei Y, Fujita M, Tanimoto K, Tsuda M, Sanada M, Ohama K, Takahashi J, Rohlin M. Dental panoramic radiograph as a tool to detect postmenopausal women with low bone mineral density: untrained general dental practitioners' diagnostic performance. Osteoporos Int 2003;14:659–664

National Institute for Alcohol Abuse and Alcoholism, United States Department of Health and Human Services, Alcohol and Health. Ninth special report to the United States on alcohol and health (NIH Publication No. 97-4017). 1997, Washington, DC, US Goverment Printing Office

Nelson DA, Megyesi MS. Sex and ethnic differences in bone architecture. Curr Osteoporos Rep 2004;2:65–69

Neugebauer J, Shirani R, Mischkowski RA, Ritter L, Scheer M, Keeve E, Zöller JE. Comparison of cone-beam volumetric imaging and combined plain radiographs for localization of the mandibular canal before removal of impacted lower third molars. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:633-642

Nicholson PHF, Lowet G, Langton CM, Dequeker J, Van Der Perre G. A comparison of time-domain and frequency domain approaches to ultrasonic velocity measurements in trabecular bone physics in medicine biology. Phys Med Biol 2996;41:2421–2435

Ong CT, Ivanovski S, Needleman IG, Retzepi M, Moles DR, Tonetti MS, Donos N. Systematic review of implant outcomes in treated periodontitis subjects. J Clin Peridontol 2008;35:438-462

Orwoll ES, Oviatt SK. Longitudinal precision of dual-energy x-ray absorptiometry in a multicenter study. The Nafarelin/Bone Study Group. J Bone Miner Res 1991;6:191–197

Parissis N, Kondylidou-Sidira A, Tsirlis A, Patias P. Conventional radiographs vs digitized radiographs: image quality assessment. Dentomaxillofac Radiol 2005;34:353-356

Park HS, Lee UJ, Jeong SH, Kwon TG. Density of the alveolar and basal bones of the maxilla and mandible. Am J Orthod Dentofacial Orhop 2008;133:30-37

Parvez S, Malik KA, Ah KS, Kim HY. Probiotics and their fermented food products are beneficial for health. J App Microbiol 2006;100:1171-1185

Patel S, Dawood A, Ford TP, Whaites E. The potential applications of cone beam computed tomography in the management of endodontic problems. Int Endod J 2007;40:818-830

Payne JB, Reinhardt RA, Nummikoski PV, Patil KD. Longitudinal alveolar bone loss in postmenopausal osteoporotic/osteopenic women. Osteoporos Int 1999;10:34–40

Payne JB, Zachs NR, Reinhardt RA, Nummikoski PV, Patil K. The association between oestrogen status and alveolar bone density changes in postmenopausal women with a history of periodontitis. J Periodontol 1997;68:24–31

Pearson J, Dequeker J, Henley M, Bright J, Reeve J, Kalender W, Laval-Jeantet AM, Rüegsegger P, Felsenberg D, Adams J. European semi-anthropomorphic spine phantom for the calibration of bone densitometers: assessment of precision, stability and accuracy. The European Quantitation of Osteoporosis Study Group. Osteoporos Int 1995;5:174–184

Pecoraro M, Zadivatan-le N, Janal M, Khocht A. Comparison of observer reliability in assessing alveolar bone height on direct digital and conventional radiographs. Dentomaxillofac Radiol 2005;34:279-284

Persson GR. What has ageing to do with periodontal health and disease. Int Dent J 2006;56(Suppl 1):240–249

Pouilles JM, Tremollieres F, Ribot C. The effects of menopause on longitudinal bone loss from the spine. Calcif Tissue Int 1993;52:340-343

Rapuri PB, Gallagher JC, Balhorn KE, Ryschon KL. Alcohol intake and bone metabolism in elderly women. Am J Clin Nutr 2000;72:1206-1213

Rawlinson A, Ellwood RP, Davies RM. An *in vitro* evaluation of a dental subtraction radiography system using bone chips on dried human mandibles. J Clin Periodontol 1999;26:138-142

Reddy MS, Jeffcoat MK. Periodontal disease progression. Curr Opin Periodontol 1993:52-59

Ribeiro-Rotta RF, Lindh C, Rohlin M. Efficacy of clinical methods to assess jawbone tissue prior to and during endosseous dental implant placement: a systematic literature review. Int J Oral Maxillofac Implants 2007;22:289-300

Richy F, Ethgen O, Bruyere O, Mawet A, Reginster JY. Primary prevention of osteoporosis: mass screening scenario or pre-screening with questionnaires? An economic perspective. J Bone Miner Res 2004;10:1955–1960

Rosa GM, Lucas GQ, Lucas ON. Cigarette smoking and alveolar bone in young adults: a study using digitized radiographs. J Periodontol 2008;79:232-244

Rosen CJ. Pathogenesis of osteoporosis. In: Cooper C & Woolf AD (eds). Osteoporosis, best practice and research compendium. 2006, Elsevier, London.

Royal Colleges of Physicians, Psychiatrists and General Practitioners. In Alcohol and the Heart in Perspective: Sensible Limits Reaffirmed. 1995, RCP, RCPsych, RCGP, London

Rushton VE, Horner K, Worthington HV. Screening panoramic radiology of adults in general dental practice: radiological findings. Br Dent J 2001;12:495-501

Rushton VE, Horner K. The use of panoramic radiology in dental practice. J Dent 1996;24:185-201

Santorini C, Ceccanti M, Diacinti D, Attilia ML, Toppo L, D'rasmo E, Romagnoli E, Mascia ML, Cipriani C, Prastaro A, Carnevale V, Minisola S. Skeletal turnover, bone mineral density and fractures in male chronic abusers of alcohol. J Endocrinol Invest 2008;31:321-326

Schuit SC, van der Klift M, Weel AE, de Laet CE, Burger H, Seeman E, Hofman A, Uitterlinden AG, van Leeuwen JP, Pols HA. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone 2004;34:195-202

Sedrine WB, Chevallier T, Zegels B, Kvasz A, Micheletti MC, Gelas B, Reginster JY. Development and assessment of the Osteoporosis Index of Risk (OSIRIS) to facilitate selection of women for bone densitometry. Gynecol Endocrinol 2002;16:245–250

Shapurian T, Damoulis PD, Reiser GM, Griffin TJ, Rand WM. Quantitative evaluation of bone density using the Hounsfield index. Int J Oral Maxillofac Implants 2006;21:290–297

Shen EC, Gau CH, Hsieh YD, Chang CY, Fu E. Periodontal status in post-menopausal osteoporosis: a preliminary clinical study in Taiwanese women. J Chin Med Assoc 2004;67:389–393

Silverman SL. Quality of life issues in osteoporosis. Curr Rheumatol Rep 2005;7:39–45

Slagter KW, Raghoebar GM, Vissink A. Osteoporosis and edentulous jaws. Int J prosthodont 2008;21:19-26

Soikkonen K, Ainamo A, Wolf J, Xie Q, Tilvis R, Valvanne J, Erkinjuntti T. Radiographic findings in the jaws of clinically edentulous old people living at home in Helsinki, Finland. Acta Odontol Scand 1994;52:229–233

Southard KA, Southard TE, Schlechte JA, Meis PA. The relationship between the density of the alveolar processes and that of post-cranial bone. J Dent Res 2000;79:964–969

Southard KA, Southard TE. Detection of simulated osteoporosis in human anterior maxillary alveolar bone with digital subtraction. Oral Surg Oral Med Oral Pathol 1994;78: 655–661

Stoppie N, Pattijn V, Van Cleynenbreugel T, Wevers M, Vander Sloten J, Naert I. Structural and radiological parameters for the characterization of jawbone. Clin Oral Implants Res 2006;17:124–133

Stoppie N, van der Waerden JP, Jansen JA, Duyck J, Wevers M, Naert I. Validation of microfocus computed tomography in the evaluation of bone implant specimens. Clin Implant Dent Relat Res 2005;7:87-94

Substance Abuse and Mental Health Administration (SAMHSA), United States Department of Health and Human Services. http://oassamhsagov/NSDUH/2k6NSDUH/tabs/Sect2peTabs1to42 htm#Tab215B2008

Taguchi A, Asano A, Ohtsuka M, Nakamoto T, Suei Y, Tsuda M, Kudo Y, Inagaki K, Noguchi T, Tanimoto K, Jacobs R, Klemetti E, White SC, Horner K, OSPD International Collaborative Group. Observer performance in diagnosing osteoporosis by dental panoramic radiographs: results from the osteoporosis screening project in dentistry (OSPD). Bone 2008;43:209-213

Taguchi A, Suei Y, Ohtsuka M, Nakamoto T, Lee K, Sanada M, Tsuda M, Ohama K, Tanimoto K, Bollen AM. Relationship between self-reported periodontal status and skeletal bone mineral density in Japanese postmenopausal women. Menopause 2005;12:144–148

Taguchi A, Suei Y, Sanada M, Ohtsuka M, Nakamoto T, Sumida H, Ohama K, Tanimoto K. Validation of dental panoramic radiography measures for identifying postmenopausal women with spinal osteoporosis. Am J Roentgenol 2004;183:1755–1760

Taguchi A, Tsuda M, Ohtsuka M, Kodama I, Sanada M, Nakamoto T, Inagaki K, Noguchi T, Kudo Y, Suei Y, Tanimoto K, Bollen AM. Use of dental panoramic radiographs in identifying younger postmenopausal women with osteoporosis. Osteoporosis Int 2006;17:387–394

Taguchi A, Suei Y, Ohtsuka M, Otani K, Tanimoto K, Ohtaki M. Usefulness of panoramic radiography in the diagnosis of postmenopausal osteoporosis in women. Width and morphology of inferior cortex of the mandible. Dentomaxillofac Radiol 1996;25:263-267

Takaishi Y, Okamoto Y, Ikeo T, Morii H, Takeda M, Hide K, Arai T, Nonaka K. Correlations between periodontitis and loss of mandibular bone in relation to systemic bone changes in postmenopausal Japanese women. Osteoporos Int 2005;16:1875–1882

Tannirandorn P, Epstein S. Drug-induced bone loss. Osteoporosis Int 2000;11:637-659

Teughels W, Newman MG, Coucke W, Haffajee AD, Van Der Mei HC, Haake SK, Schepers E, Cassiman JJ, Van Eldere J, van Steenberghe D, Quirynen M. Guiding periodontal pocket recolonization: a proof of concept. J Dent Res 2007;86:1078-1082

Teughels W, Van Essche M, Sliepen I, Quirynen M. Probiotics and oral health. Periodontol 2000 2008;47:1-37

Trouerbach WT, Steen WT, Zwamborn AW, Schouten HJ. A study of the radiographic aluminium equivalent values of the mandible. Oral Surg Oral Med Oral Pathol 1984;58:610–616

van der Stelt PF. Experimentally produced bone lesions. Oral Surg Oral Med Oral Pathol 1985;59:306-312

van der Stelt PF, Geraets WG. Computer-aided interpretation and quantification of angular periodontal bone defects on dental radiographs. IEEE Trans Biomed Eng 1991;38:334–338

van Eijden TM, van der Helm PN, van Ruijven LJ, Mulder L. Structural and mechanical properties of mandibular condylar bone. J Dent Res 2006;85:33–37

von Wowern N, Klausen B, Kollerup G. Osteoporosis: a risk factor in periodontal disease. J Periodontol 1994;65:1134–1138

von Wowern N. General and oral aspects of osteoporosis: a review. Clin Oral Investig 2001;52:71-82

Walsh TF, al-Hokail OS, Fosam EB. The relationship of bone loss observed on panoramic radiographs with clinical periodontal screening. J Clin Periodontol 1997;24:153–157

Watts NB. Osteoporotic vertebral fractures. Neurosurg Focus 2001;10:E12

Weldon E, Brocklebank LM, Nairn N, Naudi K, Ayoub A. CBCT for *in vitro* research: a practical approach for using the I-Cat®. Report of the 11th congress of the European Academy of DentoMaxilloFacial Radiology 2008;160

White S, Pharoah MJ. Oral radiology. Principles and interpretation, 5th edn (pp 316–317; pp 71-85). 2004, Mosby, St. Louis, MO

White SC, Atchison KA, Gornbein JA, Nattiv A, Paganini-Hill A, Service SK, Yoon DC. Change in mandibular trabecular pattern and hip fracture rate in elderly women. Dentomaxillofacial Radiol 2005;34:168–174

White SC, Taguchi A, Kao D, Wu S, Service SK, Yoon D, Suei Y, Nakamoto T, Tanimoto K. Clinical and panoramic predictors of femur bone mineral density. Osteoporos Int 2005;16:339–346

White SC. Oral radiographic predictors of osteoporosis. Dentomaxillofac Radiol 2002;31:84–92

Wikesjö UM, Claffey N, Egelberg J. Periodontal repair in dogs. Effect of heparin treatment of the root surface. J Clin Periodontol 1991;18:60-64

World Health Organization study group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. WHO Technical Report Series No. 843. 1994, WHO, Geneva

World Health Organization. The World Health Report 1997. Conquering suffering, enriching humanity. WHO, Geneva

Xie Q, Wolf J, Ainamo A. Quantitative assessment of vertical heights of maxillary and mandibular bones in panoramic radiographs of elderly dentate and edentulous subjects. Acta Odontol Scand 1997;55:155-161

Yamashina A, Tanimoto K, Sutthiprapaporn P, Hayakawa Y. The reliability of computed tomography (CT) values and dimensional measurements of the oropharyngeal region using CBCT: comparison with multidetector CT. Dentomaxillofac Radiol 2008;37:245-251

Yan H, Nackaerts O, Duyck J, Maes F, Jacobs R. Bone quality assessment methods based on CBCT. Clin Oral Impl Res 2009 (*In press*)

Yang J, Chiou R, Ruprecht A, Vicario J, MacPhail LA, Rams TE. A new device for measuring density of jaw bones. Dentomaxillofac Radiol 2002;31:313–316

Yoshihara A, Seida Y, Hanada N, Miyazaki H. A longitudinal study of the relationship between periodontal disease and bone mineral density in community-dwelling older adults. J Clin Periodontol 2004;31:680–684

Yun JH, Hwang SJ, Kim CS, Cho KS, Chai JK, Kim CK, Choi SH. The correlation between the bone probing, radiographic and histometric measurements of bone level after regenerative surgery. J Periodont Res 2005;40:453-460

Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–577

# Curriculum Vitae

Olivia Nackaerts (°22 October 1978) obtained her Master's degree in Speech Therapy and Audiology Sciences in 2000. Her MSc thesis dealt with articulation and myofunction in edentulous patients. Before returning to dentistry, she obtained a postgraduate degree in Media and Information Sciences in 2001. At the Department of Periodontology, K.U.Leuven, she assisted in the organization of the "11th European Association for Osseointegration Congress" until 2003. From 2003, she started working at the Oral Imaging Center, K.U.Leuven. She was involved in the Osteodent project (co-ordinated by University of Manchester) in the 5$^{th}$ European Commission Framework Programme. During 2007, she was working at Speech Therapy and Audiology, division Teaching Methodology and Practicals, while continuing her research at the Oral Imaging Center. At present, she is involved in another European project co-ordinated by the University of Manchester, under the 7$^{th}$ Framework (Euratom), called SedentexCT.

## PUBLICATIONS

### *Peer-reviewed journals*

- Manders E, Jacobs R, Nackaerts O, Van Looy C, Lembrechts D. The influence of oral implant-supported prostheses on articulation and myofunction. Acta Oto-Rhino-Laryngol Belg 2003;57:73-77
- Nackaerts O, Jacobs R, Pillen M, Engelen L, Gijbels F, Devlin H, Lindh C, Nicopolou-Karayianni K, van der Stelt P, Horner K. Accuracy and precision of a densitometric tool for jaw bone. Dentomaxillofac Radiol 2006;35:244-248
- Gijbels F, Jacobs R, Princen K, Nackaerts O, Debruyne F. Potential occupational health problems for dentists in Flanders, Belgium. Clin Oral Invest 2006;10:8-16
- Molly L, Nackaerts O, Vandewiele K, Manders E, van Steenberghe D, Jacobs R. Speech adaptation after treatment of full edentulism through immediate loaded implant protocols. Clin Oral Impl Res 2008;19:86-90
- Nackaerts O, Jacobs R, Zhao F, Lindh C, Karayianni K, van der Stelt P, Pavitt S, Horner K, Devlin H. Bone density measurements in intra-oral radiographs. Clin Oral Invest 2007;11:225-229

- Nackaerts O, Gijbels F, Sanna AM, Jacobs R. Is local bone quality as assessed on panoramic radiographs indicative of periodontal bone loss? Clin Oral Investig 2008;12:31-35
- Nackaerts O, Jacobs R, Devlin H, Pavitt S, Bleyen E, Borghs H, Lindh C, Karayianni K, van der Stelt P, Marjanovic E, Adams J, Horner K. Osteoporosis detection using intra-oral densitometry. Dentomaxillofac Radiol 2008;37:282-287
- Nackaerts O, Jacobs R, Quirynen M, Rober M, Sun Y, Teughels W. Replacement therapy for periodontitis: pilot radiographic evaluation in a dog model. J Clin Periodontol 2008;35:1048-1052
- Nackaerts O, Jacobs R, Devlin H, Horner K, Karayianni K, Mitsea A, Berkas L, Mastoris M, Lindh C, van der Stelt P, Harrison E, Adams JE, Pavitt S. Is self-reported alcohol consumption associated with osteoporotic mandibular bone loss in women? Eur J Oral Sci 2009;117:7-12
- Yan H, Nackaerts O, Duyck J, Maes F, Jacobs R. Bone quality assessment methods based on CBCT. Clin Oral Impl Res 2009 (*In press*)
- Sun Y, De Dobbelaer B, Nackaerts O, Loubele M, Yan B, Suetens P, Politis C, Vrielinck L, Schepers S, Lambrichts I, Horner K, Devlin H, Jacobs R. Development of a clinically applicable tool for bone density assessment. Int J CARS 2009 (*In press*)

## *Book Chapters*

- Nackaerts O, Jacobs R. Logopedische Screening. In: Data Dentaal. 2003, Bohn Stafleu Van Loghum, Houten, Nederland
- Nackaerts O, Jacobs R. Logopedische klachten bij rehabilitatie met orale implantaten. In: Handboek Orale Implantaten. 2003, Bohn Stafleu Van Loghum, Houten, Nederland
- Nackaerts O, Jacobs R. De link tussen logopedie en tandheelkunde. In: Tandheelkundig jaar 2006. 2006, Bohn Stafleu Van Loghum, Houten, Nederland

- Nackaerts O, Jacobs R, Gijbels F. Orale radiografie als screeningsinstrument voor osteoporose? In: Tandheelkundig jaar 2006. 2006, Bohn Stafleu Van Loghum, Houten, Nederland

## *Other publications*

- Nackaerts O, Jacobs R, Manders E. Logopedische klachten na rehabilitatie met orale implantaten. Tandartsenwereld 2003;121:5-8
- Nackaerts O, Jacobs R. Osteoporose in het kaakbot. Mondmagazine 2003;7:13
- Nackaerts O. Kan een tandarts bijdragen tot detectie van Osteoporose? Verslag 5e Edmin symposium. Tandartsenkrant 2004;290:10
- Nackaerts O. Imaging Osteoporosis. Verslag 5e EDMIN symposium, 1 april 2004. Tandartsenwereld 2004;125:4-5
- Nackaerts O, Jacobs R, Gijbels F. Osteoporosescreening met behulp van orale radiografie. Tandheelkundige Tijdingen 2005;33:77-83
- Nackaerts O, Jacobs R, Gijbels F. Kan kaakbotradiografie een bijdrage leveren tot osteoporosescreening? Tijdschrift voor Geneeskunde 2005;61:953-958
- Nackaerts O, Jacobs R. Kan orale radiografie helpen bij osteoporosescreening? Dentist News & Tandartsenwereld 2006;2:25-29
- Nackaerts O. 6e EDMIN symposium – 10th European Congress on Dentomaxillofacial Radiology. Dentist News & Tandartsenwereld 2006;6:29-30
- Nackaerts O. 6e EDMIN symposium – 10th European Congress on Dentomaxillofacial Radiology. Tandartsenkrant 2006;302:5

## *Abstracts*

- Jacobs R, Manders E, Nackaerts O, Carette E, Peeters J, Naert I, van Steenberghe D. Could there be a link between speech pathology, hearing impairment, tooth loss and oral implant placement? 23rd Conference of the Society of Oral Physiology, May 15 – 18 2003, Leuven, Belgium (Poster)
- Nackaerts O, Sanna AM, Joachims A, Du Tré F, Luyten F, Jacobs R. *In vitro* validation of mandibular bone densitometric analysis. 9th European Congress on Dentomaxillofacial Radiology, June 17 – 19 2004, Malmö, Sweden (Poster)
- Sanna AM, Jacobs R, Horbachova M, Thijs M, Nackaerts O, Gijbels F. Association periodontal disease with cardiovascular disease using panoramic

radiographs and vascular ultrasound. 9th European Congress on Dentomaxillofacial Radiology, June 17 – 19 2004, Malmö, Sweden (Poster)

- Nackaerts O, Pillen M, Engelen L, Gijbels F, Luyten F, Jacobs R. Accuracy and Precision of a Densitometric Tool for Jaw Bone. Finalist of the Research Award of the 15th International Congress on Dentomaxillofacial Radiology, May 28 – June 2 2005, Cape Town, South Africa (Oral presentation) **In**: Reports from 15th International Congress of Dentomaxillofacial Radiology. Medimond, Bologna, Italy. pp 51-55
- Sanna AM, Nackaerts O, Jacobs R, Gijbels F, van Steenberghe D. *In vitro* assessment of radiological bone density changes in the mandible. 14th Congress of the European Association of Osseointegration, September 22 – 24 2005, Munich, Germany. Clinical Oral Implants Research; 16:lxxv (Poster)
- Nackaerts O, Gijbels F, Sanna A, Abarca M, Jacobs R, Slaets B. Colapso periodontal y su relación con la densidad ósea en radiografías panorámicas. 6° Encuentro Latinoamericano de Radiologia Oral y Maxilo Facial, April 28 – May 1 2006, Santiago, Chile (Oral presentation)
- Nackaerts O, Jacobs R, Devlin H, Lindh C, Karayianni K, van der Stelt P, Harrison E, Adams J, Pavitt S, Horner K. ROC analysis of densitometric measurements on intra-oral radiographs for detection of osteoporosis as part of the OSTEODENT study. 10th European Congress on Dentomaxillofacial Radiology, June 1 – 3 2006, Leuven, Belgium (Oral presentation) **In**: Report of the 10th European Congress of Dentomaxillofacial Radiology. p 46
- Sanna AM, Gijbels F, Nackaerts O, Horbachova M, Baltekenova N, Jacobs R. Detection of carotid atheromata and other soft tissue calcifications on panoramic radiographs of a hospital population. 10th European Congress on Dentomaxillofacial Radiology, June 1 – 3 2006, Leuven, Belgium (Poster) **In**: Report of the 10th European Congress of Dentomaxillofacial Radiology. p 126
- Sun Y, de Dobbelaer B, Nackaerts O, Loubele M, Suetens P, Jacobs R. Development of a preoperative bone quality assessment tool using digital 2D and 3D: decision of design parameters. 10th European Congress on Dentomaxillofacial Radiology, June 1 – 3 2006, Leuven, Belgium (Poster)

**In**: Report of the 10th European Congress of Dentomaxillofacial Radiology. p 113

- Teughels W, Nackaerts O, Quirynen M, Jacobs R. Replacement therapy for periodontitis: bone density changes in the dog model. 16th International Congress on Dentomaxillofacial Radiology, June 26 – 30 2007, Beijing, China (Oral presentation)
- Nackaerts O, Hua Y, Duyck J, Jacobs R. Bone quality assessment method based on CBCT technology. 11th Congress of the European Academy of Dentomaxillofacial Radiology, June 25 – 28 2008, Budapest, Hungary (Poster)
- Sun Y, De Dobbelaer B, Nackaerts O, Loubele M, Yan B, Suetens P, Politis C, Vrielinck L, Schepers S, Lambrichts I, Horner K, Devlin H, Jacobs R. Validation of a clinically applicable tool for bone density assessment. 11th Congress of the European Academy of Dentomaxillofacial Radiology, June 25 – 28 2008, Budapest, Hungary (Oral presentation)