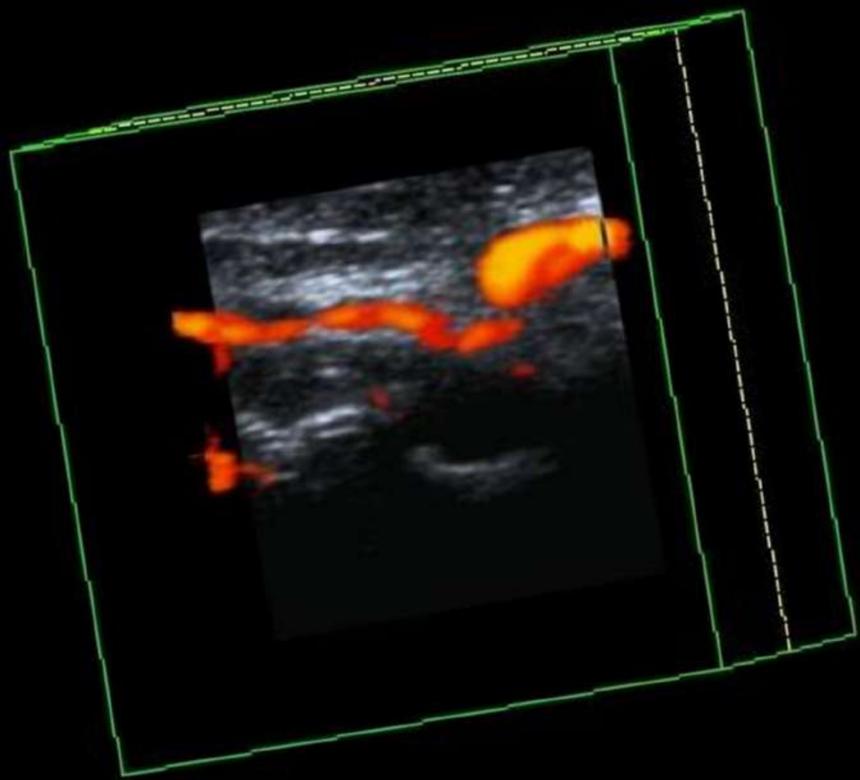
Clinical applications of ultrasound examination in the assessment of bone lesions of the jaws



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The clinical applications of ultrasound examination in the assessment of bone lesions of the jaws

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Chapter 1

Introduction

Dental diagnostics and treatment planning strongly rely on imaging techniques, and outcome assessment is usually based on clinical examination and radiographs. Orthopantomography and intraoral radiographs, which are captured using X-ray films or digital sensors, are routinely used for these purposes, but they have significant limitations because they compress 3-dimensional (3D) anatomy into two dimensions, causing geometrical distortion and anatomical noise (Nardi *et al.* 2017, Patel *et al.* 2009a). To overcome these limitations, alternative imaging modalities have been explored (Cotti & Campisi 2004). Cone beam computed tomography (CBCT) was introduced as an upgrade to traditional CT scanners. It exhibits higher resolution and is therefore a promising tool for diagnosing dental problems. Furthermore, most CBCT devices involve much lower radiation exposure than traditional CT; they are simple to use and are roughly the same size as panoramic machines, so they are suitable for dental settings (Patel *et al.* 2009a, Scarfe *et al.* 2006). However, the quality and diagnostic accuracy of CBCT images are affected by scatter and beam-hardening artifacts caused by high-density structures such as enamel and radiopaque materials used in the dental field (Shaha *et al.* 2014). In addition, CBCT exposes the patient to ionizing radiation, so it should be avoided routinely for diagnostic or screening purposes (Patel *et al.* 2019, Silva *et al.* 2008). Magnetic resonance imaging (MRI) does not use ionizing radiation, and it is mainly used in dentistry to investigate the salivary glands and temporomandibular joint (Patel *et al.* 2009a). In a recent study, MRI was used to assess intraosseous lesions of the jaws because it can distinguish solid from liquid material in a lesion, as well as epithelium (Lizio *et al.* 2018). However, MRI and CBCT cannot detect the vascularity of a lesion without contrast enhancement (Lizio et al.2008). Moreover, strong magnetic fields may not be safe in patients with pacemakers, implantable defibrillators, artificial heart valves, cerebral aneurysm clips, and similar devices (Shaha *et al.* 2014). MRI has poorer resolution than simple radiographs, as well as longer scanning times and higher hardware costs. Finally, it can only be accessed in dedicated radiology units (Patel *et al.* 2009a).

Ultrasound examination, also called ultrasonography, real-time echography, or echo-tomography, is a real-time imaging modality that has been used in many fields of medicine for more than 50 years (AIUM 2015). It is a non-invasive procedure because no access through the skin is needed, and there is no direct contact with the mucosa or internal body cavities (Haar 2011). In addition, it is considered a reliable, cost-effective, and safe imaging technique with no epidemiological evidence of harmful effects at normal diagnostic levels (Duck 2001, Moore 2011, Salvesen 2002). Despite their excellent safety record, ultrasound imaging techniques discharge mechanical energy, which subsequently interacts with vital tissues. For this reason, different studies have investigated the possible negative effects of ultrasound (Abramowicz 2002, Miller 1991, WFUMB 1998). To date, no harmful mechanical or thermal effects of ultrasound examination have been demonstrated in humans using the frequencies and exposure time necessary in clinical practice (Brown 1984, Carstensen 1987, Duck 2001 ,Nyborg 1992, Moore 2011, Salvesen 2002). However, exposure to ultrasound should be kept as low as reasonably achievable (ALARA). Ultrasound imaging should only be performed when clinically necessary by trained professionals who understand how to use the modality safely (Haar 2011).

Ultrasound examination does not use ionizing radiation. Instead, it is based on the propagation and reflection of ultrasound waves into and from the tissues of the body. Therefore, while X-rays can pass freely through a vacuum, ultrasound waves require a medium that can transmit mechanical energy (Kumar & Mahabob 2010). Ultrasound waves are generated through a transducer or probe when an alternating electric current is applied to a crystal of quartz or composite piezoelectric material. As a result of the piezoelectric effect, the transducer produces acoustic ultrasound waves oscillating at the same frequency. When ultrasound waves encounter biological tissues of different densities that have distinct mechanical and acoustic properties, a portion of the wave is deflected back to the transducer by reflection or scattering. The residual mechanical energy is transmitted through the tissues. The echo is the part of the ultrasound wave that is deflected towards the crystal. When ultrasound waves return to the crystal, they receive and transmit sound accordingly, with the changes in thickness indicated by the conversion of the sound waves into electrical current and subsequently into grayscale images on a computer screen (Cotti *et al.* 2002, Goldstein 1993). Early ultrasonography used a single crystal to create a 1D scan line called the "A mode" (amplitude mode), with the echo amplitude displayed vertically and the echo time of flight displayed horizontally (Girish & Jacobson 2002).

The standard screen image currently generated by ultrasound machines is called the "B mode" (brightness mode), in which electrical energy is transformed into a light spot using a grayscale on a monitor. The ultrasonographic image seen on the monitor is generated by the automatic movement of the crystal over the tissues examined, producing a scan line that is used to create an image or frame. In the B mode, the real-time image is refreshed many times per second—at least 16 times per second with modern devices—to produce a moving image on the screen. An indicator on the probe is used to guide the user in determining the orientation of the plane on the screen. The operator uses the controls on the console to optimize the system for different anatomic targets (Girish & Jacobson 2009). The frequency of diagnostic ultrasound is measured in MHz. Ultrasound waves generated at lower frequencies have better penetration, but lower resolution, while those generated at higher frequencies produce images with a higher resolution at the expense of penetration into the anatomical structures (Moore & Copel 2011). The examined tissues can be echogenic (i.e., generating echoes) or anechoic\transonic (i.e., not generating echoes because no reflection occurs within those tissues). Echogenic tissues can be defined as hyperechoic—showing high echo intensity—when a strong reflection occurs at the interfaces of organs and tissues. They are defined as hypoechoic when the tissue interface reflection has a low intensity. Fluid-filled cavities are normally anechoic, while areas with the same acoustic properties as the surrounding tissues are isoechoic (Cotti & Campisi 2004, McDicken& Anderson 2002). Ultrasound examination can be enhanced using the Doppler mode to measure and visualize perfusion of the tissues. The Doppler mode produces a real-time, 2D image of vascularity as a color overlay on a B-mode scan. Two types of Doppler imaging are possible: color Doppler and power Doppler imaging. Color Doppler imaging provides information on the mean velocity of blood cells at a given time, producing images on a color scale. Typically, red is used to indicate flow towards the transducer, while blue denotes flow away from the transducer. Power Doppler imaging measures the number of moving cells in the sample volume. It has greater sensitivity than color Doppler to depict flow in small vessels. In both modalities, low flow velocities are displayed as dark shades of red or blue, whereas high-flow velocities are displayed as lighter shades, according to a scale (McDicken& Anderson 2002). Traditionally, volume within the body was studied by moving the ultrasonographic probe over the area of interest, changing the anatomical plane, and thus providing a real-time, 3D impression of the space. However, this procedure requires a highly experienced sonographer and lacks accuracy when reproducing 3D structures (Girish & Jacobson 2009). Subsequently, 3D ultrasound imaging was developed to help clinicians acquire a full understanding of the spatial anatomical relationships. Using this modality, B-scans are collected using either conventional 2D probes or dedicated 3D probes. The 2D images are then converted into a volume that is visualized on the screen in real-time or near real-time (Huang & Zeng 2016).

Ultrasound was first applied in dentistry in 1963 by Baum *et al.*, who demonstrated that the internal structures of vital teeth, including enamel, dentin, and pulp tissue, could be distinguished (Baum *et al.* 1963). Recent findings have confirmed that general-purpose sonographic machines can image dentin, cementum, and dental pulp spaces *in vivo* through the cervical area of teeth, which is not covered by bone or prosthetic crowns (Szopinski & Regulski 2014). As such, tooth integrity can be determined using ultrasound, and proximal carious lesions can be diagnosed with a high level of accuracy *in vitro* (Tagtekin *et al.* 2008 and Matalon *et al.* 2007). A later *in vivo* study confirmed that ultrasound imaging can be used to evaluate cavitated proximal carious lesions, with a minimum reduction in accuracy (Matalon *et al.* 2007). The same principles were used to detect dental cracks and fractures in both dental phantom models and extracted natural teeth (Culjat *et al.* 2005, Singh *et al*. 2007).

Ultrasound examination can also be used to assess periodontal tissues. An *ex vivo* study on pig mandibles demonstrated that high-frequency ultrasound imaging could accurately and consistently measure the periodontal structures, including the distance between the cementum-enamel junction and the alveolar bone crest; the technique was compared to transgingival probing and direct measurements, which are normally considered the gold standard (Tsiolisis *et al.* 2003). These findings were later confirmed in studies that compared ultrasound images with CBCT and direct microscopy section measurements (Chifor *et al.* 2011, Nguyen *et al.* 2016). Another study (Mahmoud *et al.* 2010) detected periodontal anatomical landmarks, including the cementum-enamel junction, root, tooth cervical area, and coronal edge of cortical bone. The same authors measured experimental bony defects in both the horizontal and vertical components. Furthermore, ultrasound imaging has been applied in periodontology to measure soft tissue parameters, such as gingival thickness, and to distinguish among different periodontal phenotypes. Clinical studies on humans have confirmed that ultrasound examination can determine gingival thickness at the mid-buccal surfaces rather than at the interproximal papilla, where measurements are unreliable (Müller & Könönem 2005, Savitha & Vandana 2005). Increased reliability of ultrasound imaging was shown *in vivo* when the gingiva was thinner. Gingival appraisal resulted to be better in dental sites related to upper canines, first premolars, lower anterior teeth, and premolars, whereas it was poorly reproducible in the lower third molars, where the gingiva is much thicker (Müller *et al.* 2007). Consequently, reduced reliability is expected in gingivitis, in which inflammatory infiltration causes swelling.

Ultrasound examination has not been completely validated in implant dentistry, but a recent systematic review clarified its potential technique during the three phases of implant treatment: planning, intraoperative, and postoperative (Bhaskar *et al.* 2018). In the planning phase, echography can be used to evaluate vital structures, such as the greater palatine foramen, mandibular lingual foramen, and lingual nerve, which are difficult to identify using current clinical methods. It can also identify tissue biotypes, ridge width/density, and cortical bone thickness. During surgery, it can provide feedback on the bone boundary and vital structures, such as the inferior alveolar nerve and maxillary sinus. At follow-up visits, it can be used to evaluate the level of marginal bone and implant stability. In addition, in pig models, ultrasound imaging can be used to measure soft tissue thickness over bone, as well as implants that are submerged in bone beneath soft tissue (Culjat *et al.* 2008).

Conversely, ultrasound examination has found wider application in the evaluation of oral soft tissue diseases (Marotti *et al.* 2013). It is a valuable diagnostic tool to confirm infectious disease in the superficial facial spaces, and it is highly reliable when predicting stage of infection (Rama Moan *et al.* 2015). The ultrasonographic appearance of abscesses varies from anechoic to irregularly hyperechoic due to internal echoes made by sediments. Abscesses range in shape from round and generally well-defined to irregular and lobulated, and they can be examined in motion when palpated or pressed. Cellulitis is usually characterized by tissues that are more hyperechoic than normal because of massive inflammatory infiltration (Khartik *et al.* 2016). Conversely, osteomyelitis involves juxta-cortical soft tissue swelling, periosteal thickening, subperiosteal exudate, and cortical erosion on ultrasound (Rama Mohan *et al.* 2015). Ultrasound examination has also been applied to soft tissue malignancies in the oral cavity. A review article pointed out that intraoral ultrasound with color and power Doppler imaging can estimate the depth of invasion of tongue carcinoma, as well as its thickness, extent, and microvascular proliferation, which predict the tumor's ability to spread to the lymph nodes and metastasize (Hayashi 2012).

Considering that ultrasonography can provide information on the size, tissue characteristics, and vascularity of lesions, it could be applicable to intra-osseous lesions of the jaws. This is an intriguing possibility, because such lesions have a high incidence, involve complex differential diagnosis, and are not easily or readily accessible (Philipsen *et al.* 2017). A 1996 study was the first study to apply echographic examination to jaw lesions detected using panoramic, occlusal, and periapical radiographs. The results demonstrated that 90% of the lesions could be visualized using standard equipment. The remaining 10% of the lesions could not be seen because they had small diameters or were associated with a thicker cortical plate. Some inconclusive cases involved lesions that contained mineralized tissue, such as ossifying fibromas and dentigerous cysts, which can act as confounders when interpreting sonographic images (Dib *et al.* 1996). This protocol was subsequently reproduced using color-power Doppler to implement grayscale B-mode imaging. The accuracy of the sonographic diagnosis was 95% compared to the gold standard of histological examination (Sumer *et al.* 2009).

In endodontics, ultrasound examination was introduced for the first time in 2002, when Cotti *et al.* demonstrated that clinicians could obtain echographic images of apical periodontitis (AP) and collect information related to its content (Cotti *et al.* 2002). In later studies, this technique was enhanced by color and power Doppler to differentiate between granulomatous and cystic apical lesions, using histopathology as a reference standard (Cotti *et al.* 2003, Cotti *et al*. 2006). These results were later confirmed using the same protocol (Marotti *et al.* 2013). Some studies have attempted to standardize the application of echography to monitor periapical bone healing after surgical and non-surgical endodontic therapy (Rajendran & Sundaresan 2007, Curvers *et al.* 2017). However, a systematic review of the literature must be carried out to summarize the current scientific evidence.

One limitation of echography applied to jawbone lesions is that it cannot easily visualize dental landmarks (such as dental morphology) in the B-mode on a screen. However, such landmarks can be seen while performing the examination directly on the patient, and they can be visualized clinically during real-time scanning of the patient, but not once the image has been printed or saved onto a hardware (Cotti et al. 2002). Another limitation is that it is difficult for untrained physicians or dentists to interpret echographic images (Patel *et al*. 2009a, Shah *et al.* 2014), although there is no evidence that a lack of training results in unreliable interpretation of ultrasound images of intra-bony lesions among dental professionals (Marotti et *al.* 2013). Moreover, training in this technique could be introduced to dental programs once it becomes more prominent.

Conflicting information has been reported regarding how difficult it is to assess intra-bony lesions in the posterior maxilla and mandible. Some authors have stated that posterior access is limited because conventional transducers are used or because the bony plate is thicker in those areas (Gundappa *et al.* 2006, Tikku *et al.* 2016). Conversely, other studies have visualized lesions in both the posterior and anterior sites with equal success (Cotti *et al.* 2003, Zainedeen *et al.* 2018). Furthermore, some studies have argued that perforation or consistent erosion of one cortical plate must be present before a lesion within the jawbones can be identified using ultrasonography (Patel *et al*. 2009a, Rajendran & Sundaresan 2007), although there is little evidence to support this claim.

Objectives of the thesis

The aim of this thesis was to investigate whether real-time ultrasound examination could be used to assess bone lesions affecting the jaws. In addition, we assessed whether the technique could differentiate between periapical lesions of endodontic origin and other pathological conditions. Finally, we investigated the healing of periapical lesions after endodontic treatment.

Outline of the thesis

Chapter 2 discusses the clinical case that inspired the subsequent chapters. Two different pathological entities, namely a traumatic bone cyst and apical periodontitis, arose within the same area. A multi-modular approach was used, in which color and power Doppler ultrasound was the only imaging modality that displayed the features later confirmed by histopathological examination.

Chapter 3 presents a systematic review of the literature, which was carried out to collect the best available evidence regarding the diagnostic accuracy of ultrasound imaging for intraosseous lesions of the jaws, with histopathological examination as a gold standard.

Chapter 4 describes a clinical study that investigated whether color-power Doppler ultrasound examination of the early vascular response to treatment in the affected area could be used to predict the healing of apical periodontitis after endodontic therapy. The predictions were correlated with long-term clinical and radiological assessment.

Chapter 5 analyzed real-time ultrasound examination to detect sinus tracts of endodontic origin, tracing the drainage route from the periapical lesion to the opening within the oral mucosa or skin.

Chapter 6 reports an *ex vivo* study that aimed to address the main concerns about the use of ultrasound to assess lesions in the jaws. In particular, it focused on whether cortical plate integrity could influence the images obtained through the exam. Artificial bony defects were created to evaluate the accuracy of this technique when diagnosing lesions with incomplete or limited erosion of the cortical bone plate, as well as to determine the minimum cortical thickness that constitutes a barrier for ultrasound waves.

Chapter 7 is a systematic review of the literature aimed at understanding the state of the art in the use of color and power Doppler ultrasound to monitor the healing of intraosseous bone lesions following treatment.

References

Abramowicz JS (2002) Ultrasound in obstetrics and gynecology: is this hot technology too hot? *Journal of Ultrasound in Medicine* **21**, 1327–33.

American Institute of Ultrasound in Medicine (2015) The AIUM celebrates 50 years of excellence. Available at: http://www.aium.org/ aboutUs/history/articles/jum1.pdf.

Baum G, Greenwood I, Slawski S, Smirnow R (1963) Observation of internal structures of teeth by ultrasonography. *Science* **139**, 495–6.

Bhaskar V, Chan HL, MacEachern M, Kripfgans OD (2018) Updates on ultrasound research in implant dentistry: a systematic review of potential clinical indications. *Dentomaxillofacial Radiology* **47**, 1-13.

Brown BSJ (1984)How safe is ultrasonography? *Canadian Medical Association* **131**, 307-11.

Carstensen EL (1987) Acoustic cavitation and the safety of diagnostic ultrasound. *Ultrasound in Medicine & Biology* **13**, 597-606.

Chifor R, Hedesiu M, Bolfa P, Catoi C, Crisan M, Serbanescu A, et *al*. (2011) The evaluation of 20 MHz ultrasonography, computed tomography scans as compared to direct microscopy for periodontal system assessment. *Medical Ultrasonography* **13**, 120-6.

Cotti E, Campisi G, Garau V, Puddu G (2002) A new technique for the study of periapical bone lesions: ultrasound real time imaging. *International Endodontic Journal* **35**, 148-152.

Cotti E, Campisi G, Ambu R, Dettori C (2003) Ultrasound real-time imaging in the differential diagnosis of periapical lesions. *International Endodontic Journal* **36**, 556-63.

Cotti E, Campisi G (2004) Advanced radiographic techniques for the detection of lesions in bone. *Endodontic Topics* **7**,52-72.

Cotti E, Simbola V, Dettori C, Campisi G (2006) Echographic evaluation of bone lesions of endodontic origin: report of two cases in the same patient. *Journal of Endodontics* **32**, 901-5.

Culjat MO, Singh RS, Brown ER, Neurgaonkar RR, Yoon DC,White SN (2005) Ultrasound crack detection in a simulated human tooth. *Dentomaxillofacial Radiology* **34**, 80-5.

Culjat MO, Choi M, Singh RS, Grundfest WS, Brown ER, White SN (2008)Ultrasound detection of submerged dental implants through soft tissue in a porcine model. *Journal of Prosthetic Dentistry* **99**, 218-24.

Curvers F, Meschi N, Vanhoenacker A, Strijbos O, Van Mierlo M, Lambrechts P (2018) Ultrasound Assessment of Bone Healing after Root-end Surgery: Echoes Back to Patient's Safety. *Journal of Endodontics* **44**,32-7.

Dib LL, Curi MM, Chammas MC, Pinto DC, Torloni H (1996) Ultrasonography evaluation of bone lesions of the jaw. *Oral Surgery Oral Medicine Oral Pathology* **82**, 351-7.

Duck FA (2011) Recent epidemiology papers: European committee for medical ultrasound safety. *Ultraschall in der Medizine* **13**, 227-31.

Girish G, Jacobson JA (2009) Ultrasound. In: Weissman BN, ed. Imaging of Arthritis and Metabolic Bone Disease. Philadelphia, PA: Saunders; pp. 89-103.

Goldstein A (1993) Overview of the physics of US. *Radiographics* **13**, 701-4.

Gundappa M, Ng SY, Whaites EJ (2006) Comparison of ultrasound, digital and conventional radiography in differentiating periapical lesions. *Dentomaxillofacial Radiology* **35**, 326-33.

Hayashi T (2012) Application of Ultrasonography in dentistry. *Japanese Dental Science Review* **48**, 5-13.

Haar TG (2011) Ultrasonic imaging: safety considerations. *Interface Focus* **1**, 686-97.

Huang Q, Zeng Z (2017) A Review on Real-Time 3D Ultrasound Imaging Technology. *BioMed Research International* **x**, 1-20.

Karthik R, Mohan N, Ravikumar PT, Fenn SM, Gokulraj S, Cicilia Subbulakshmi C (2016) Ultrasonography in maxillofacial Imaging – a review. *International Journal of Maxillofacial Imaging* **2**, 63-8.

Kumar BS, Mahabob MN (2010) Ultrasound in dentistry e a review. *Journal of Indian Academy of Dental Specialist* **1**, 44-5.

Lizio G, Salizzoni E, Coe M, Gatto M, Asioli S, Balbi T, Pelliccioni GA (2018) Differential diagnosis between a granuloma and radicular cyst: effectiveness of magnetic resonance imaging. *International Endodontic Journal* **51**, 1077-87.

Mahmoud AM, Ngan P, Crout R, Mukdadi OM (2010) High-resolution 3D ultrasound jawbone surface imaging for diagnosis of periodontal bony defects: an in vitro study. *Annals of Biomedical Engineering* **38**, 3409-3422.

Marotti J, Heger S, Tinschert J, et *al.* (2013) Recent advances of ultrasound imaging in dentistry: a review of the literature. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology* **115**, 819-32.

Martin K (2011) Basic equipment, components and image production. In: Allan PL, Baxter GM, Weston M, eds. Clinical Ultrasound. 3rd ed. St. Louis, MO: Churchill Livingstone; pp. 16-30.

Matalon S, Feuerstein O, Kaffe I (2003) Diagnosis of approximal caries: bite-wing radiology versus the ultrasound caries detector. An in vitro study. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontology* **95**, 626-31.

Matalon S, Feuerstein O, Calderon S, Mittleman A, Kaffe I (2007) Detection of cavitated carious lesions in approximal tooth surfaces by ultrasonic caries detector. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontology* **103**, 109-13.

McDicken WN, Anderson T (2002) The difference between colour Doppler velocity imaging and power Doppler imaging. *European Journal of Echocardiography* **3**, 240-4.

Miller DL (1991) Update on safety of diagnostic ultrasonography. *Journal of Clinical Ultrasounds* **19**, 531-40.

Moore CL, Copel JA (2011) Point-of-care ultrasonography. *New England Journal of Medicine* **364**, 749-57.

Muller HP, Kononen E (2005) Variance components of gingival thickness. *Journal of Periodontal Research* **40**, 239-44.

Muller HP, Barrieshi-Nusair KM, Kononen E (2007) Repeatability of ultrasonic determination of gingival thickness. *Clinical Oral Investigation* **11**, 439-42.

Nguyen K-CT, Le LH, Kaipatur NR, Zheng R, Lou EH, Major PW (2016) High-Resolution Ultrasonic Imaging of Dento-Periodontal Tissues Using a Multi-Element Phased Array System. *Annals of Biomedical Engineering* **x**, 1-13.

Nyborg WL (1992) Scientifically based safety criteria for ultrasonography. *Journal of Ultrasound in Medicine* **11**, 425-32.

Patel S, Dawood A, Whaites E, Pitt Ford T (2009a) New dimensions in endodontic imaging: part 1. Conventional and alternative radiographic systems. *International Endodontic Journal* **42**, 447-62.

Patel S, Dawood A, Mannocci F, Wilson R, Pitt Ford T (2009b) Detection of periapical bone defects in human jaws using cone beam computed tomography and intraoral radiography. *International Endodontic Journal* **42**, 507-15.

Patel S, Brown J, Semper M, Abella F, Mannocci F (2019) European Society of Endodontology position statement: Use of cone beam computed tomography in Endodontics: European Society of Endodontology (ESE). *International Endodontic Journal* **52**, 1675-78.

Philipsen HP, Reichart PA, Slootweg PJ, Slater LJ (2017) Odontogenic and maxillofacial bone tumours. In: El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg P, editors. *WHO classification of Head and Neck Tumours.* 4th edn Lyon: IARC; pp. 205-60.

Rajendran N, Sundaresan B (2007) Efficacy of ultrasound and color power Doppler as a monitoring tool in the healing of endodontic periapical lesions. *Journal of Endodontics* **33**, 181-6.

Rama Mohan K, Koteswara Rao N, Leela Krishna G, Santosh Kumar V, Ranganath N, Vijaya Lakshmi U (2015) Role of ultrasonography in oral and maxillofacial surgery: a review of literature. *Journal of Maxillofacial and Oral Surgery* **14**, 162-70.

Salvesen KA (2002) Epidemiology of diagnostic ultrasound exposure during pregnancy. *Ultraschall in der Medizine* **15**, 165-71.

Savitha B, Vandana KL (2005) Comparative assessment of gingival thickness using transgingival probing and ultrasonographic method. *Indian Journal of Dental Research* **16**, 135-9.

Scarfe WC, Farman AG, Sukovic P (2006) Clinical applications if cone-beam computed tomography in dental practice. *Journal of the Canadian Dental Association* **72**, 75–80.

Shah N, Bansal N, Logani A (2014) Recent advances in imaging technologies in dentistry. *World Journal of Radiology* **6**, 794-807.

Silva MA, Wolf U, Heinicke F, Bumann A, Visser H, Hirsch E (2008) Cone-beam computed tomography for routine orthodontic treatment planning: a radiation dose evaluation. *American Journal of Orthodontics and Dentofacial Orthopedics* **133**, 640.e1-640.e5.

Singh RS, Culjat MO, Cho JC, et *al.* (2007) Penetration of radiopaque dental restorative materials using a novel ultrasound imaging system. *American Journal of Dentistry* **20**, 221-6.

Sumer AP, Danaci M, Sandikci EO, Sumer M, Celenk P (2009) Ultrasonography and Doppler ultrasonography in the evaluation of intraosseous lesions of the jaws. *Dentomaxillofacial Radiology* **38**, 23-27.

Szopinski KT, Regulski P (2014) Visibility of dental pulp spaces in dental ultrasound *Dentomaxillofacial Radiology* **43**, 1-6.

Tagtekin DA, Ozyoney G, Baseren M, et *al.* (2008) Caries detection with DIAGNOdent and ultrasound. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontology* **106**, 729-35.

Tikku AP, Bharti R, Sharma N, Chandra A, Kumar A, Kumar S (2016) Role of ultrasound and color doppler in diagnosis of periapical lesions of endodontic origin at varying bone thickness. *Journal of Conservative Dentistry* **19**, 147-5

Tsiolis FI, Needleman IG, Griffiths GS (2003) Periodontal ultrasonography. *Journal of Clinical Periodontology* **30**, 849–54.

WFUMB (1998) Symposium on Safety of Ultrasound in Medicine. Conclusions and recommendations on thermal and non-thermal mechanisms for biological effects of ultrasound. Kloster-Banz, Germany. 14–19 April,1996. World Federation for Ultrasound in Medicine and Biology. *Ultrasound in Medicine & Biology* **24**, x-x.

Zainedeen O, Haffar IA, Kochaji N, Wassouf G (2018) The efficacy of ultrasonography in monitoring the healing of jaw lesions. *Imaging Science in Dentistry* **48**, 153-160.

Chapter 2

Multimodular Assessment of a Traumatic Bone Cyst Overlapped with Apical Periodontitis

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Case Report

Multimodular Assessment of a Traumatic Bone Cyst Overlapped with Apical Periodontitis

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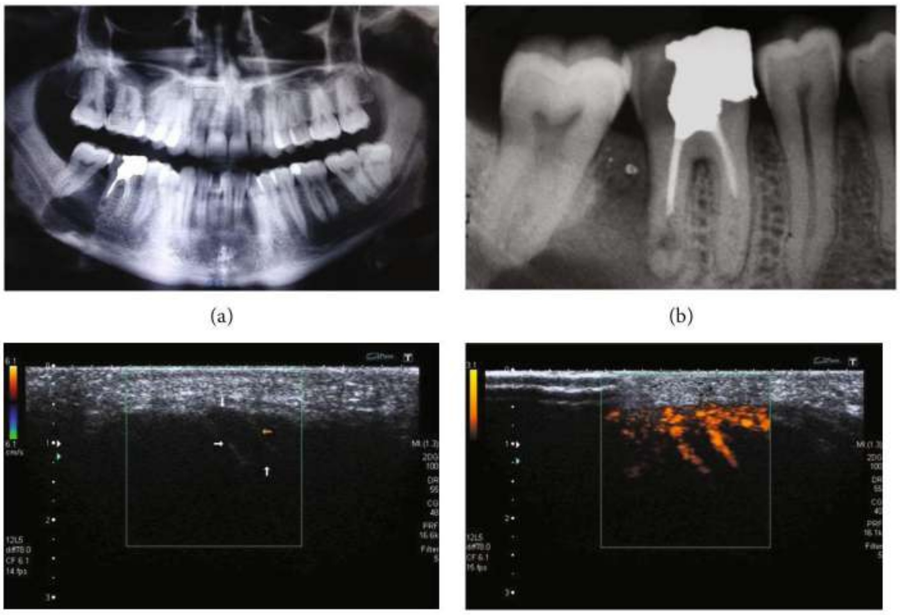
Traumatic bone cyst (TBC), a "pseudocyst" that usually affects long bones, is a rare lesion among cystic lesions in the jaws. The most commonly affected site is the posterior mandible. Most of the time, TBC is asymptomatic and discovered during routine radiographic examination. The treatment recommended for TBC is surgical exploration followed by curettage of the bony walls, which also serves as a diagnostic procedure. A 27-year-old Caucasian male with a noncontributory medical history was referred to our department for the endodontic evaluation of the mandibular right first and second molars, which were connected to an extensive asymptomatic osteolytic lesion. A multimodular diagnostic assessment involving CBCT imaging, ultrasound, and histopathologic examination led to a definite diagnosis of a TBC overlapping with apical periodontitis (AP). Subsequently, a multidisciplinary treatment approach was performed, including surgical excision and biopsy of the lesion, endodontic retreatment of the right mandibular first molar, and postsurgical root canal treatment of the second molar. During the follow-up period of five years, the patient was reassessed periodically once a year and showed, in the absence of signs and symptoms, progressive healing of the affected area. The present article reports a case following the CARE guidelines of a TBC combined with AP where a multimodular diagnostic assessment was performed and discusses the possible pathogenetic mechanisms involved in its generation.

1. Introduction

Although the majority of osteolytic lesions in the periradicular area of teeth are inflammatory in origin, some may not be inflammatory. The assessment of osteolytic lesions in the maxillary bones should always involve an exhaustive medical history report and a careful clinical examination comprising diagnostic tests and radiographic examinations that can be crucial in the differential diagnosis between apical periodontitis (AP) and nonendodontic lesions [1]. Three-dimensional imaging systems provide additional information on the extension of the lesions in the maxillary bones, their relationships with the surrounding anatomical structures and the aggressiveness of the disease, [2] while ultrasound examination with color-power Doppler can detect the content of the pathologic cavity (i.e., solid and empty/fluid filled) and its vascular supply [3]. Traumatic bone cysts (TBCs) are rare lesions that constitute 0.2 to 0.9% of all cystic lesions in the jaws, where the site most commonly affected is the posterior mandible. It is often diagnosed during the first two decades of life with an even distribution among the sexes [4]. TBC usually affects long bones and is defined as "an intraosseous cyst having a tenuous lining of connective tissue with no epithelium" and consequently a "pseudocyst" [5]. Most of the time, TBC is asymptomatic and discovered during routine radiographic examination; alternative pain is the most common symptom, together with tooth sensitivity, paresthesia, and painless swelling. Despite its name, a clear history of trauma in TBC is often questionable [5, 6]. The treatment recommended for TBC is surgical exploration followed by curettage



(a) (b) (c) (d)

Figure 1: Images of the 27-year-old patient presenting an extensive asymptomatic lesion of the right posterior mandible. (a) The panoramic radiograph shows a well-defined unilocular osteolytic lesion with sclerotic margins located between the two molar teeth and superimposed on the alveolar inferior nerve. (b) The periapical radiograph of the first molar depicting inadequate root canal treatment with signs of perforation in the apical third of the distal root involved in the osteolytic lesion. (c) B-mode ultrasound examination showing a transonic lesion (arrows). (d) Power-Doppler image showing the presence of peripheral vascularity.

of the bony walls, which also serves as a diagnostic procedure [4–7]. The purpose of the present article is to report a case following the CARE guidelines [8] of a TBC combined with AP, where a multimodular diagnostic assessment was performed and to discuss the possible pathogenetic mechanisms involved in its generation.

2. Case Presentation

A 27-year-old Caucasian male with a noncontributory medical history and no previous trauma was referred from the maxillofacial department of the hospital for the endodontic evaluation of the mandibular right first and second molars, which were connected to an extensive asymptomatic osteolytic lesion incidentally discovered on a routine panoramic radiograph and scheduled to be treated surgically under general anesthesia. The patient reported that he had experienced pain and swelling in the right mandible months before, while the first molar had a history of caries, extensive amalgam restoration, and incongruous endodontic treatment performed ten years earlier. The clinical examination showed no sign of buccal or lingual bone expansion, no lymph node involvement, and intact overlaying mucosa. The first mandibular molar was asymptomatic. The right second mandibular molar was asymptomatic with an intact crown, and it responded normally to the sensitivity tests at the time of our examination. The panoramic radiograph showed a well-defined unilocular osteolytic lesion with sclerotic margins located between the two molar teeth and superimposed on the alveolar inferior nerve with a slight displacement of the second molar. The first molar showed inadequate root canal treatment with signs of resorption in the apical third of the distal root involved in the osteolytic lesion. A preoperative periapical radiograph taken using the paralleling technique confirmed these findings (Figure 1). Cone beam computed tomography (CBCT) revealed a well-defined unilocular lesion, with considerable expansion toward the lingual wall and consequent thinning of the lingual plate. From the axial and sagittal sections, it was possible to diagnose a perforation both in the distal and lingual aspects of the apical third of the distal root of the right first molar (Figure 2). To assess the content and vascularity of the lesion, a real-time ultrasound examination with the application of color-power-Doppler (CPD) was performed using a Toshiba Aplio XG (Toshiba Medical Systems, Crawley, UK) apparatus with a regular size, linear, high definition, and multifrequency ultrasound probe at 8-12 MHz. The exam displayed a transonic, fluid-filled lesion with a well-defined hyperechoic bone contour, perilesional vascularity, and no internal vascular supply, suggestive of a cystic lesion (Figure 1). The diagnosis, based on all the exams performed, was a nonendodontic cystic lesion of the right mandible in the area of the first and second mandibular molars and AP in a previously treated first mandibular molar with a perforation in the distal root. The treatment plan was discussed with the patient and comprised the following steps: (1) surgical excision and biopsy of the lesion for the histopathologic evaluation; (2) endodontic retreatment of the right mandibular first molar, with the repair of the perforating defect on the distal root; (3) a possible postsurgical root canal treatment of the second

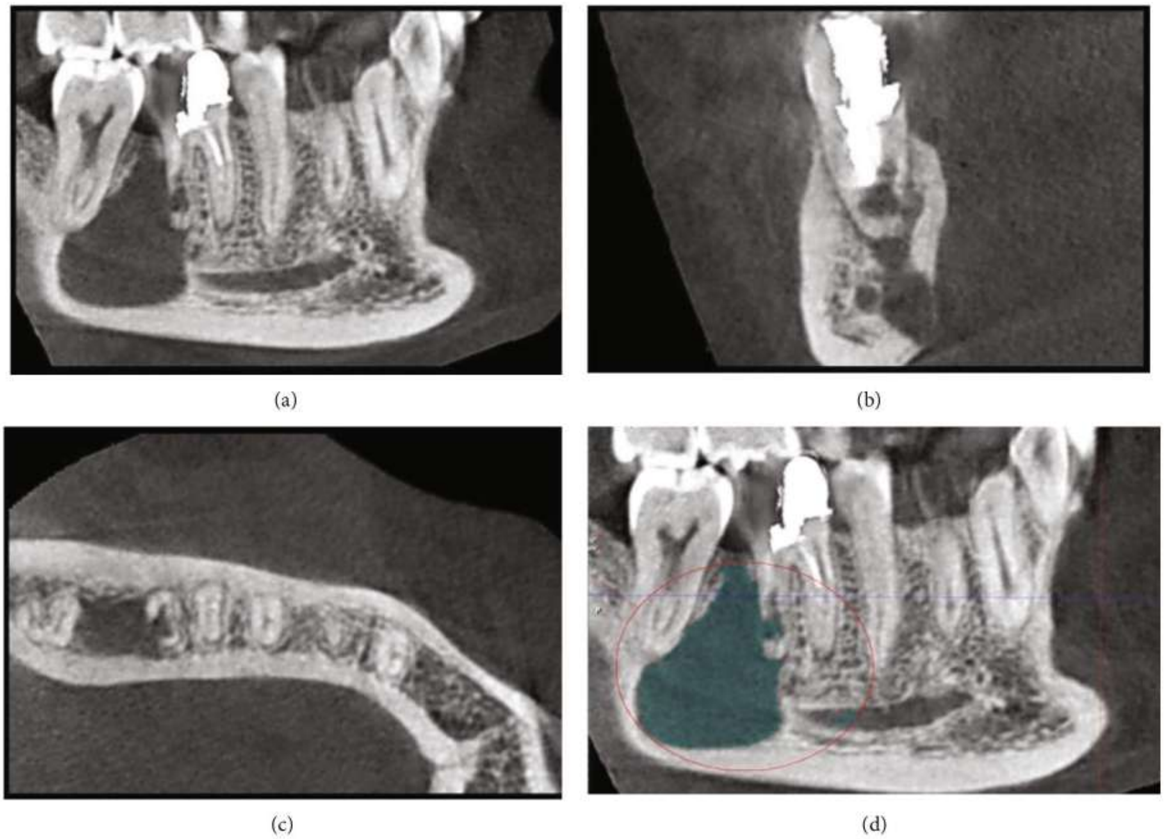


FIGURE 2: (a) CBCT revealing a well-defined unilocular lesion between the second and first right mandibular molars. (b) Sagittal view showing the expansion of the lesion toward the lingual wall and the consequent thinning of the lingual plate. (c) Axial section showing a perforation in the distal and lingual aspects of the apical third of the distal root of the right first molar. (d) The main osteolytic lesion appears in continuity with the perforation in the distal root of the right mandibular first molar.

molar. Surgery was performed in the maxillofacial clinic under general anesthesia after an inferior alveolar nerve block together with infiltration with anesthetic of the surrounding tissues. A mucoperiosteal flap was raised exposing the buccal bone, which did not present any sign of resorption or expansion. A bone window was created using a surgical bur with continuous water cooling to reach the lesion (Figure 3); the disclosed bone cavity had the size and shape shown by CBCT and was filled with serum/blood fluid, as seen in the ultrasound exam; thus, a temporary intraoperative diagnosis of TBC was made. The fluid was evacuated, and the walls of the lesion were carefully explored and curetted; however, only a few specimens of tissue were collectable for histopathologic examination. After the formation of the blood clot, the flap was repositioned and sutured. Histologic examination of the tissue fragments reported fibrosclerotic tissue with calcifications and cholesterol clefts, extensively interested in a chronic inflammatory infiltrate with numerous foamy histiocytes and foreign body–like multinucleated giant cells (Figure 3). Four weeks later, the patient was asymptomatic; however, at the postsurgical endodontic assessment, the second molar did not respond to the sensitivity tests, which was due to a possible resection of the alveolar neurovascular bundle, and a diagnosis of pulp necrosis was made. After informed consent was obtained, local anesthesia was administered using mepivacaine 2% 1 : 100 000 epinephrine followed by isolation of the teeth using a rubber dam. Root canal treatment of the second molar was performed in 1 appointment without complications. Two weeks later, the patient was still asymptomatic, and secondary treatment was performed on the first molar. After informed consent, local anesthesia administration, rubber dam isolation, and root canal retreatment of the mesial canals were achieved without obstacles, while the working length determined with the apex locator in the distal root canal was considerably shorter than the length of the root because of perforation, which was established by measuring the CBCT scans. The canals were instrumented manually and irrigated with 5.25% NaOCl and a final rinse of 17% EDTA solution. The apical half of the distal canal was dried with paper points and obturated using Ortho-MTA (BioMTA, Seoul, Republic of Korea) applied with a carrier to seal the perforating defect, while the remaining coronal half was filled with flowable gutta-percha and the tooth restored with composite (Figure 4). Four months later, the patient was asymptomatic, and radiographic control showed an increase in radiopacity and trabeculae formation in the bone (Figure 4). The patient attended the periodical

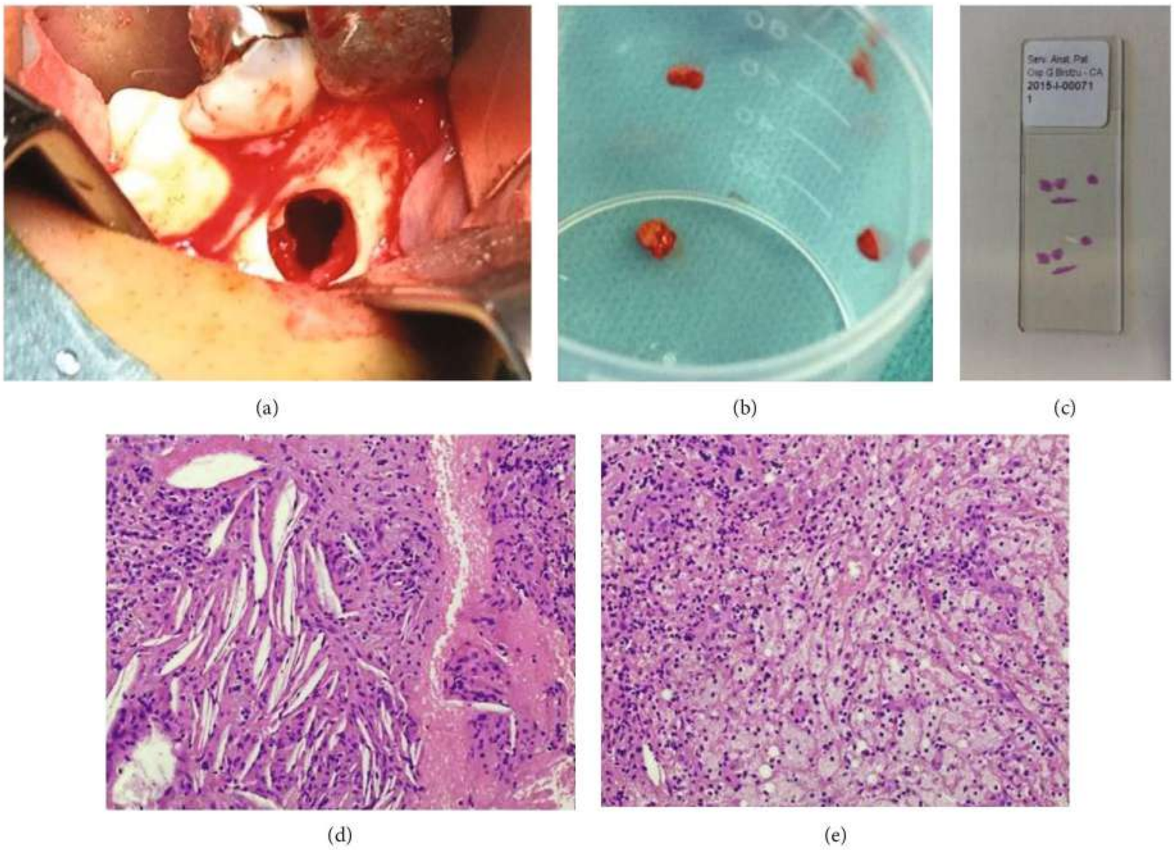


Figure 3: (a) Intraoperative view showing the bone cavity filled with serum sanguineous fluid. (b) Gross specimens of small dimensions collected for histopathological examination. (c) Hematoxylin-eosin histology slide containing the tissue fragments. (d) Photomicrograph shows the lesion to be composed of fibrosclerotic tissue with calcifications and cholesterol clefts and the absence of epithelium. (e) Histopathological section revealing the presence of a chronic inflammatory infiltrate with numerous foamy histiocytes and multinucleated giant cells.

recall visits, and at the five-year follow-up was asymptomatic. The radiographic examination showed complete healing of the lesion, with remineralization of the area around and within the perforated root. An additional CBCT examination depicted the complete formation of the vestibular and lingual cortical plates (Figures 4 and 5).

3. Discussion

The present report documents a case of a mandibular lesion diagnosed as TBC accompanied by complications, such as involvement in the field of a tooth affected by AP and an inflammatory granuloma-like histological pattern. TBC is described with a variety of names (simple bone cyst, hemorrhagic bone cyst, solitary bone cyst, extravasation cyst, and unicameral bone cyst), suggesting a lack of complete understanding of its nature [9]. Indeed, several possible theories have been formulated for its etiology and pathogenesis [6, 10–12], as reported in Table 1. The radiographic features of a TBC are those of a unilocular, well-circumscribed, radiolucent lesion with or without sclerotic margins, which may extend between the roots of teeth that are normally vital with the lamina dura preserved [7, 9]. Our CBCT findings were coherent with these descriptions, except for the loss of the lamina dura around the distal root of the first molar, a finding that validated the diagnosis of AP on that tooth. Through ultrasound real-time examination with CPD, the lesion was visualized as cystic (transonic/anechoic well-defined cavity with no evidence of central vascularization and a strong peripheral power Doppler signal to indicate a layer of vascular lining in the walls of the lesion) [13], findings that were confirmed following surgical exploration and biopsy. To our knowledge, this is the first time a TBC was assessed by ultrasound examination. According to the literature, during surgical exploration, the TBC appears as a cavity that can be either empty or filled with fluid (serum or blood), and a small amount of fibrous connective tissue can be collected occasionally while curetting the walls [9, 12, 14, 15]. Histopathologic examination usually reveals the absence of epithelial lining, presence of connective tissue, areas of vascularity with occasional chronic inflammatory cells, and scattered areas of new bone formation [14, 15]. In the present case, the small fragments collected showed highly vascularized connective tissue, confirming the echographic data. Nevertheless, the pathological analysis highlighted an inflammatory transformation of the connective wall of the lesion

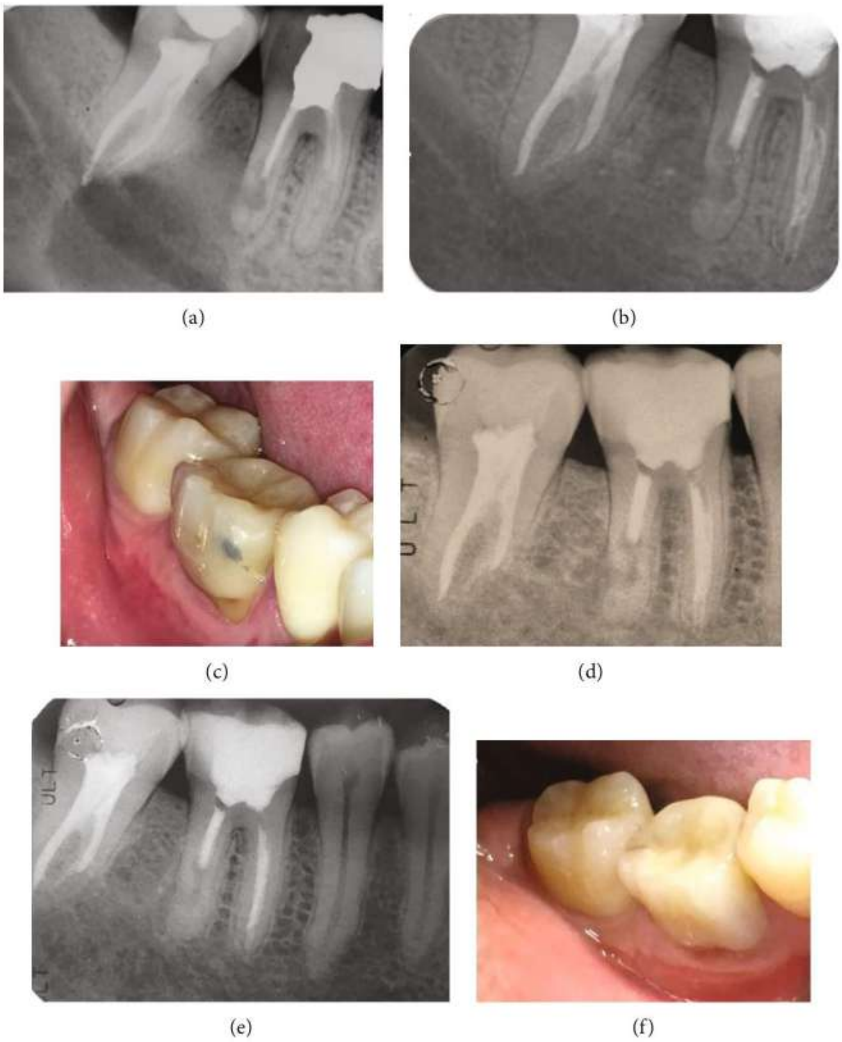
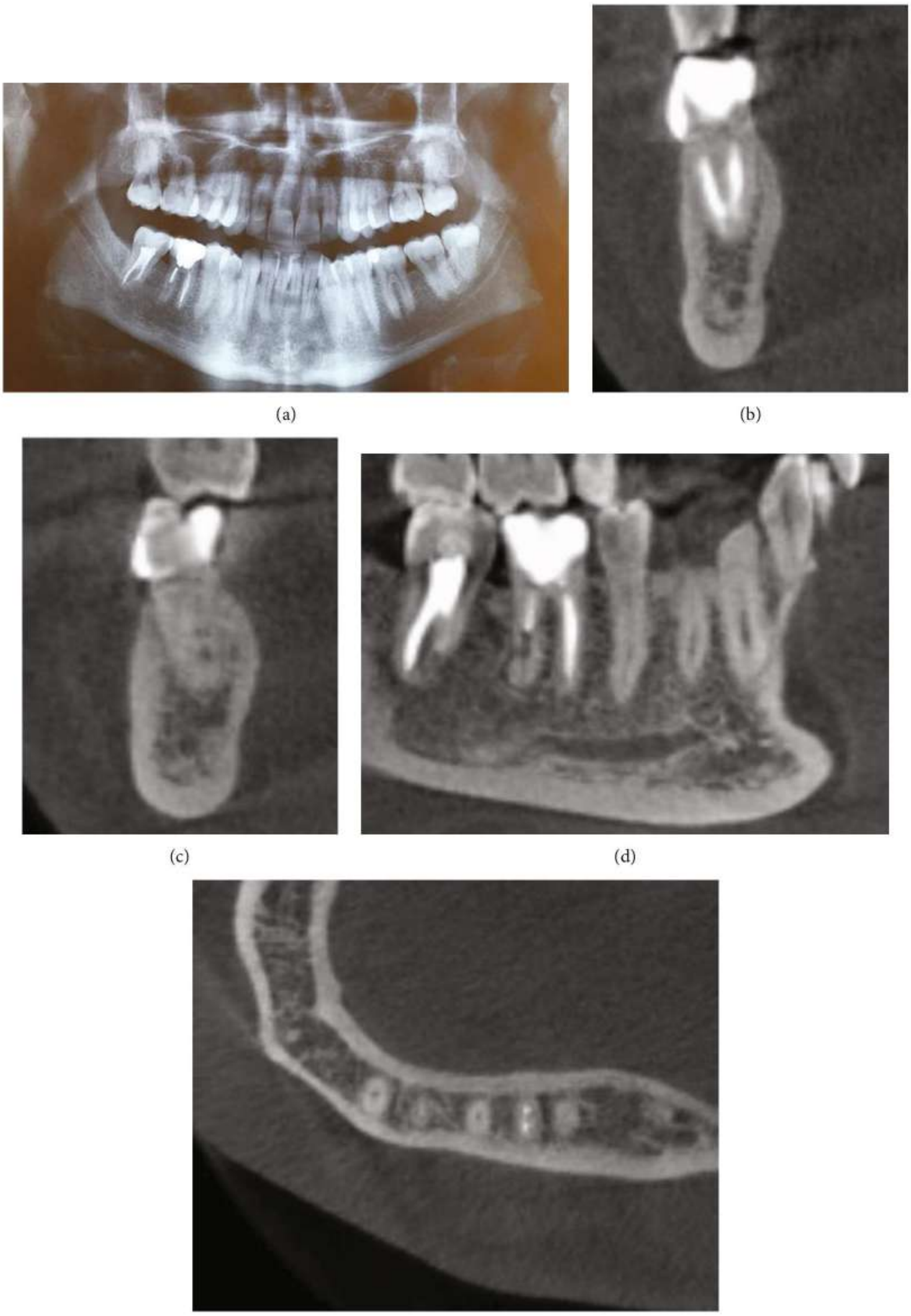


Figure 4: (a) Postoperative periapical radiograph showing root canal treatment of the second mandibular molar. (b) Four-month periapical radiograph showing an increase in radiopacity and trabeculae formation in the area of the intervention. (c) Four-month clinical photograph. (d) Two-year follow-up periapical radiograph. (e) Five-year follow-up periapical radiograph showing both repair of the distal root and bone healing. (f) Clinical photograph at the five-year recall visit.

indistinguishable from that observed for an apical granuloma. While the cause and progression of TBC remain unclear (Table 1), trauma is still the most important etiological factor and must be considered in the greater varieties of clinical situations [6, 10–12, 15]. According to this case, different hypotheses should be considered in the pathogenesis of this lesion. First, the TBC and AP developed independently; the first was due to one of the theories presented [1–6] and the latter was due to infection of the root canal system. The contemporary expansion of the two lesions in the mandible and the resorption of the surrounding bone created communication between lesions, which may have maintained their specificities. The second hypothesis is supported by the first theory reported in Table 1. AP developed initially in the first molar and during root canal treatment of the tooth, iatrogenic perforation (trauma) generated intraosseous bleeding (theory 1). According to a third hypothesis, the low-grade, chronic infection sustained by the right mandibular first molar may have influenced the bone marrow in the vicinity, which is involved in the later pathogenesis of TBC (theory 2). A limitation of the present report relies on the decision to treat the second molar, which was due to a postsurgical diagnosis of pulp necrosis following cold sensitivity tests. Although the testing was performed four weeks after surgery, it is possible for the pulpal neural bundle to await, to require more time to recover. Fortunately, upon access to the pulp chamber, the tissues appeared ischemic, and the clinical diagnosis was confirmed. The strength of the present report was the multimodular assessment of the case where the radiographs disclosed the presence of the lesions and the previous dental treatments. The content and the features of the TBC were evaluated with echography, while CBCT was of paramount importance to determine the volume and spatial relationship of the lesion with and within the anatomical landmarks to plan the endodontic and surgical interventions and to achieve a predictable follow-up.



(a) (b) (c) (d) (e)

Figure 5: Panoramic radiograph showing complete healing and absence of recurrence after five years. (b–d) Five-year CBCT examination showing complete healing of the lesion. (c–e) The sagittal and axial views demonstrate remineralization of the area around and within the perforated root and depict the complete formation of the vestibular and lingual cortical plates.

Furthermore, the composite treatment plan involving the surgical access of the TBC and the endodontic retreatment of the right mandibular first molar affected by AP resulted in very satisfactory healing.

4. Conclusions

The clinical significance of this case report is the presence of two lesions, AP and TBC, combined in the same anatomical

Table 1: Etiopathogenetic theories of the TBC.

N°	Theory	Statement
1	Traumatic-hemorrhagic	A trauma can lead to intramedullary hemorrhage. Compromised vascular supply, edema, and aseptic bone necrosis caused by the trauma lead the blood clot to liquefy. In the area, lytic enzymes are released, and osteoclastic bone resorption is activated. The expansion of the cavity seems to be sustained by the edema and blood extravasation.
2	Infective	A small, low-grade, chronic infection of bone marrow is involved in the pathogenesis
3	Lesion degeneration	A developing tumor or lesion (i.e., hemangioma, lymphoma, fibrous-osseous dysplasia, or central giant cell granuloma) undergoes liquid degeneration, leaving behind an empty cavity.
4	Local thrombosis	A local thrombosis can generate either a local ischemia with necrosis of bone marrow and blockage of interstitial fluid drainage leading to the formation and expansion of an intraosseous cavity.
5	Developmental	A failure of mesenchymal tissue to form bone and cartilage occurs and instead generates immature synovial cavities, which coalesce to form a larger connective tissue-lined defect.
6	Systemic disease	An imbalance between osteoclastic and osteoblastic activity, parathyroid disease, or a peculiarity in vessel walls or blood coagulation can predispose to the development of a TBC.

site, and such presentation has not been described before in the literature. The management of this pathological entity possibly was due to a multimodular assessment. In addition, this is the first report to describe the ultrasound examination of a combined lesion.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Consent

Informed consent was obtained from the patient for being included in the study.

Conflicts of Interest

The authors deny any conflicts of interest related to this paper.

References

[1] C. D. Rodrigues and C. Estrela, "Traumatic bone cyst suggestive of large apical periodontitis," *Journal of Endodontia*, vol. 34, no. 4, pp. 484–489, 2008.

[2] D. MacDonald, "Lesions of the jaws presenting as radiolucencies on cone-beam CT," *Clinical Radiology*, vol. 71, no. 10, pp. 972–985, 2016.

[3] D. Musu, G. Rossi-Fedele, G. Campisi, and E. Cotti, "Ultrasonography in the diagnosis of bone lesions of the jaws: a systematic review," *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, vol. 122, no. 1, pp. e19–e29, 2016.

[4] J. J. Kuttemberg, M. Farmand, and H. Stöss, "Recurrence of a solitary bone cyst of the mandibular condyle in a bone graft," *Oral Surgery, Oral Medicine, and Oral Pathology*, vol. 74, no. 5, pp. 550–556, 1992.

[5] L. Barnes, J. W. Eveson, P. Reichart, and D. Sidransky, "Pathology and Genetics of Head and Neck Tumours," in *WHO Classification of Tumours*, IARC Press, Lyon, 3rd edition, 2005.

[6] A. A. Xanthinaki, K. I. Choupis, K. Tosios, V. A. Pagkalos, and S. I. Papanikolaou, "Traumatic bone cyst of the mandible of possible iatrogenic origin: a case report and brief review of the literature," *Head & Face Medicine*, vol. 12, pp. 32–40, 2006.

[7] P. J. Chapman and K. Romaniuk, "Traumatic bone cyst of the mandible; regression following aspiration," *International Journal of Oral Surgery*, vol. 14, no. 3, pp. 290–294, 1985.

[8] J. J. Gagnier, G. Kienle, D. G. Altman, D. Moher, H. Sox, and D. Riley, "The CARE guidelines: consensus-based clinical case reporting guideline development," *Headache*, vol. 53, no. 10, pp. 1541–1547, 2013.

[9] Y. Suei, A. Taguchi, and K. Tanimoto, "A comparative study of simple bone cysts of the jaw and extracranial bones," *Dento Maxillo Facial Radiology*, vol. 36, no. 3, pp. 125–129, 2007.

[10] K. Nilesh, A. V. Vande, S. Tewary, and K. V. Suresh, "Traumatic bone cyst of an anterior mandible with previous symphyseal fracture in a pediatric patient: a rare finding and etiopathologic correlation," *General Dentistry*, vol. 65, no. 6, pp. e5–e8, 2017.

[11] S. Bindra, G. Jadaun, H. S. Jois, and P. Sen, "Traumatic bone cyst of mandible: a case report of rare entity and review of literature," *Contemporary Clinical Dentistry*, vol. 10, pp. 3–8, 2019.

[12] J. C. Harnet, T. Lombardi, P. Klewansky, J. Rieger, M. H. Tempe, and J. M. Clavert, "Solitary bone cyst of the jaws: a review of the etiopathogenic hypotheses," *Journal of Oral and Maxillofacial Surgery*, vol. 66, no. 11, pp. 2345–2348, 2008.

[13] E. Cotti and G. Campisi, "Advanced radiographic techniques for the detection of lesions in bone," *Endodontic Topics*, vol. 7, no. 1, pp. 52–72, 2004.

[14] P. R. Martins-Filho, S. Santos Tde, V. L. Araújo, J. S. Santos, E. S. Andrade, and L. C. Silva, "Traumatic bone cyst of the mandible: a review of 26 cases," *Brazilian Journal of Otorhinolaryngology*, vol. 78, no. 2, pp. 16–21, 2012.

[15] D. Musu and E. Cotti, "Traumatic bone cyst of the jaws: an overview," *Journal of Biological Regulators and Homeostatic Agents*, vol. 33, no. 4, pp. 1261–1263, 2019.

Chapter 3

Ultrasonography in the diagnosis of bone lesions of the jaws: a systematic review

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Ultrasonography in the diagnosis of bone lesions of the jaws: a systematic review

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The diagnostic use of ultrasonography in dentistry and maxillofacial surgery has previously been described in the literature. Considering that ultrasonography may be useful for the diagnosis of bone lesions of the jaws, a systematic review was carried out to examine the evidence. This review determined that ultrasonography has been used effectively for the diagnosis of infective and/or inflammatory lesions, cysts, nonodontogenic tumors, odontogenic tumors, and arteriovenous malformations and for the differential diagnosis of lesions of endodontic origin, compared with the gold standard of histologic analysis. Ultrasonography may be a viable adjunct to other special tests for the diagnosis of intraosseous lesions of the jaws, as it is noninvasive and does not involve ionizing radiation exposure of the patient. (Oral Surg Oral Med Oral Pathol Oral Radiol 2016;122:e19-e29)

Ultrasonography, also called real-time echography or tomography, is an imaging technique based on the propagation and reflection of ultrasound waves into the tissues of the body. Since the 1950s, it has become widely used in several fields of medicine.[1] Ultrasound waves are generated through a transducer by a crystal (i.e., quartz or composite piezoelectric material) when an alternating electric current is applied. As a result of the piezoelectric effect, the transducer produces acoustic ultrasound waves oscillating at the same frequency. When ultrasound waves encounter biologic tissues with different densities and mechanical and acoustic properties, a portion is deflected (reflected and/or scattered) back to the transducer, and the remainder of the mechanic energy is transmitted through the tissues. The echo is the part of the ultrasound wave that is reflected back toward the crystal. When ultrasound waves return to the crystal, they receive and transmit sound accordingly, with the changes in thickness indicated by conversion of the sound waves into electrical current and subsequently into gray-scale images on a computer screen. Early ultrasonography used a single crystal to create a one-dimensional image called "A mode" (amplitude mode), with the echo amplitude being displayed vertically and the echo time of flight being displayed horizontally. The standard screen image currently generated by ultrasound machines is called "B mode" (brightness mode), with electrical energy transformed into a light spot using a gray-scale on a monitor. The ultrasonographic image seen on the monitor is produced by means of the automatic movement of the crystal over the tissues examined, producing a scan line that is used to create an image or frame. In the B mode, the real-time image is refreshed many times per second (with modern tools at least 16 scans per second) to produce a moving image on the screen. Moving the ultrasonographic probe over the area of interest changes the anatomic plane and thus provides a real-time three-dimensional impression of the space. An indicator on the probe is used to guide the user in determining the orientation of the plane on the screen. The operator uses the controls available on the console to optimize the system operation for different anatomic targets.[2-6]

The frequency of diagnostic ultrasound is in MHz, with lower-frequency ultrasound waves having better penetration but at a lower resolution, and higher-frequency ultrasound waves producing images with a higher quality resolution at the expense of penetration into deep structures.[5,6]

The examined tissues can be echogenic (i.e., they generate echoes) or anechoic or transonic (i.e., they do not generate echoes because no reflection occurs within those tissues). Echogenic tissues can be defined as *hyperechoic* (appearing as brighter features in the image) when a strong reflection occurs at the interfaces of organs and tissues. For example, tissue−bone interfaces produce a strong reflection that will be intact if the bone

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Statement of Clinical Relevance

This systematic review presents possible clinical applications of ultrasonography in the diagnosis of intraosseous lesions of the jaws. The analysis of the literature confirms its role in the differential diagnosis between lesions of various natures.

structure has not been partially altered by a pathologic process. Echogenic tissues are *hypoechoic* when the tissue interface reflection has a low intensity. Weaker echoes are also produced by the scattering effect from small scale features within tissues. Fluid-filled cavities are normally anechoic. Areas with the same acoustic properties of the surrounding tissues are isoechoic.[2-7]

Ultrasonographic examination can be enhanced with a Doppler system, which employs the Doppler effect to perform a detailed study of the perfusion of the tissues, producing a real-time two-dimensional image of vascularity as a color-overlay on a B-mode image. Two types of Doppler imaging are available: color-Doppler and power-Doppler imaging. Color-Doppler imaging provides information on the mean velocity of blood cells at a given time, producing images on a color scale, typically using red to indicate the flow toward the transducer and blue for the flow away from the transducer. Low-flow velocities are displayed as dark shades of red or blue, whereas high-flow velocities are displayed as lighter shades, according to a velocity scale. Power-Doppler imaging is a measure of the number of moving cells in the sample volume and has a greater sensitivity to depict flow in small vessels.[3,7,8]

Dentistry and maxillofacial surgery are strongly reliant on radiographic examination, primarily to obtain a diagnosis and subsequently to provide adequate information for treatment planning and follow-up of lesions in the jaw. However, conventional radiologic examinations, including computed tomography, especially if not used in conjunction with contrast media, provide clinicians with limited information on the content and vascular supply of lesions while exposing patients to ionizing radiation. In contrast, in addition to being a safe imaging technique, since ultrasonography does not use ionizing radiation,[9-14] it can provide information about the size, tissue characteristics, and vascularity of bone lesions. This aspect of ultrasonography seems to play a major role in the development of a preliminary differential diagnosis of bone defects of pathologic origin in the jaw.[15,16] This becomes more important when the lesions are not easily or rapidly accessible. Nevertheless, to date, ultrasonography has not become a standard examination of choice in dentistry and maxillofacial surgery.

No systematic review has been previously performed on this topic. Although two narrative review papers have been published previously,[15,16] neither focused specifically on the potential for ultrasonography in the diagnosis of intraosseus lesions. The aim of this systematic review is to analyze the efficacy of ultrasonography for the differential diagnosis of lesions in the jaw, using histopathology evaluation as the gold standard reference, to define the potential of this tool for oral and maxillofacial regions.

MATERIALS AND METHODS

A systematic review was conducted following the principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[17] and the Institute of Medicine's guidelines. The PICO (P: Patient, I: Ultrasonography, C: Histology, O: Diagnosis) question generated for the present study to guide the systematic review was: "In patients with jaw bone lesions, how closely can ultrasonography generate a diagnosis compared with histology?"

Search strategy

For the present study, the following electronic databases were used for the systematic research: PubMed's Medline, Scopus, EMBASE, BIOSIS Citation Index. The keywords "ultrasonography," "ultrasound," "bone," "lesions," "periapical," "cyst," "tumour," "jaw," "mandible," and "maxilla" were used for the research, according to the following research algorithm: {(Ultrasonography\Ultrasound) +[(Bone + Lesions + Diagnosis) OR (Cyst+Tumour)] + (Jaw\Mandible\Maxilla)] OR [(Periapical + Lesions)]}. The research algorithm generated the electronic research syntaxes that were applied to each database. The papers to be examined were collected from July 28, 2015, to August 10, 2015. Textbooks, review articles, and the references listed in papers included in the research were screened as additional sources to obtain a more complete report.

Two of the authors (D.M. and G.R.F.) independently evaluated the records obtained through the electronic search to remove duplicates and screen the records for eligibility according to inclusion and exclusion criteria. Possible contingent discrepancies were solved through discussion with a third author (E.C.) and consensus. Articles were included in the systematic review if they met the following inclusion criteria: articles in English without limits concerning the date of publication; clinical trials or reports performed in vivo in humans, using ultrasonography as a diagnostic tool; and papers that included the investigation of intraosseous lesions of the jaws and a histopathologic report or diagnosis of the lesion screened. Research articles in other languages, studies performed in animals or in vitro, and studies without a histopathologic report or diagnosis of the lesions detected with ultrasonography were excluded.

The following information was extracted for each study using a data collection sheet: author, year of publication, paper, sample size, study design, ultrasonography results, histology analysis, outcome of the study, and statistical analysis. In articles with some of the data not published, we requested the information from the corresponding author.

Quality assessment of the studies

Two authors (D.M. and G.R.F.) investigated the quality of the eligible studies. For the present systematic review, case series and case reports were included. Case series and case reports are considered low in the hierarchy of evidence; however, as far as new topics in medicine are concerned, they can be considered powerful contributions to the literature if the authors had reported the objectives and inherent limitations of their studies.[18]

The critical appraisal for case reports was assessed by using the CAse REports (CARE) guidelines checklist.[19] A scoring system was established, and papers with scores of 4 or less were classified as having low-level information quality, papers with scores of 4 to 8 were classified as having moderate-level information quality, and papers with scores of 8 or greater were classified as having high-level information quality.

Quality appraisal of the case series was performed by evaluating directness of evidence (DE) and risk of bias (RB). DE was calculated by considering the following: number of lesions, anatomic site, ultrasonography, histology, and outcome. A scoring system was established: DE was considered low when the score was 2 or less, moderate when the score was 2 to 4, and high when the score was 4 or greater. RB was calculated by using the Cochrane Collaboration's tool, modified according to the needs of this systematic review. Papers with scores of 2 or less were considered as having low RB, papers with scores of 2 to 4 were considered as having moderate RB, papers with score 4 or greater were considered as having high RB.

A qualitative synthesis of the results was performed, and statistical analysis or meta-analysis was performed if the study design of the papers was considered homogeneous and data were available for statistical analysis.

RESULTS

A total of 704 records were identified through electronic searches (503 from PubMed's MEDLINE, 59 from Scopus, 116 from Embase, and 26 from BIOSIS citation index). The titles were screened by the authors to remove duplicates; 504 duplicates were removed, and the resulting 197 abstract records were evaluated by two of the authors (D.M. and G.R.F.) to select the full-text articles for eligibility. According to exclusion criteria, 133 were excluded; 64 full-text papers were assessed for eligibility, and a total of 41 were eliminated. An additional paper was rejected because of an unexplained duplicated image from a previously published study. According to the inclusion criteria, 22 full-text articles were included for the qualitative synthesis. The references of the articles selected were cross-screened, and five relevant full-text articles were identified and included for qualitative synthesis. Textbook searches were performed, but they produced no results. Data were extracted by two of the authors independently (D.M. and G.R.F.), according to a data extraction sheet prepared previously and discussed. Missing data were requested from the authors of the articles reviewed, and unreported raw data were calculated by the authors. The papers revealed heterogeneity in terms of the design of the study, protocol of the ultrasound examination, assessment of the lesion, and statistic data. They were then divided into the three main groups presented in the tables.

Quality assessment of the studies

Quality of information in case reports was scored according to the CARE guidelines and using the CARE checklist. One paper showed low-level information quality, four papers showed moderate-level information quality, and seven papers showed high-level information quality.

Quality appraisal of the case series was carried out using DE and RB assessments. Six studies showed high DE, six others showed moderate DE, and three studies showed low DE. Eleven studies showed low RB, three studies showed moderate RB, and only one study showed high RB. Despite the presence of studies with low DE and studies with moderate to high RB, the majority of the studies showed low RB and high to moderate DE.

The literature contains very few reports on the ultrasonographic study of bone lesions and, for the most part, consists of case reports and case series. For this reason, all 15 case series and 11 case reports that satisfied the inclusion and exclusion criteria were included in the review to provide the best available and inclusive evidence.

Systematic results

The clinical trials attempting a differential diagnosis in cases of apical periodontitis showed homogeneity in the aim, study design, and in both ultrasonography and histology protocols (i.e., differential diagnoses in cases of apical periodontitis). The amount of raw data and elaborate statistical data made it possible to evaluate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for all endodontic studies, which are shown in Table I. The study by Cotti et al.[20] is a case report, which fulfills the criteria for inclusion in the apical periodontitis case series. In all of the studies, information about the sample size and the frequency (MHz) used for the ultrasound examination were reported, and in general, the ultrasonic probes were used with low frequency.

Table I. Data summary of the studies with lesions of endodontic origin (in chronologic order)

Study	Study design	Age range (y)	Sample (n)	Teeth	Location	Frequency (MHz)	Sensitivity US PG	Specificity US PG	PPV US PG	NPV US PG	Sensitivity US PC	Specificity US PC	PPV US PC	NPV US PC	Accuracy US
Cotti et al., 2003[23]	CS	25-50	11	Anterior/posterior	Maxilla/mandible	7-9	100%	100%	1.00	1.00	100%	100%	1.00	1.00	100%
Gundappa et al., 2006[26]	CS	13-40	15	Anterior	Maxilla/mandible	8-11	100%	100%	1.00	1.00	100%	100%	1.00	1.00	100%
Cotti et al., 2006[20]	CR	40	2	Anterior	Maxilla	7-9	100%	100%	1.00	1.00	100%	100%	1.00	1.00	100%
Raghav et al., 2010[25]	CS	15-45	21	Anterior	Maxilla/mandible	8-11	100%	92.9%	0.88	1.00	92.3%	100%	1.00	0.89	95.2%
Prince et al., 2011[22]	CS	13-65	15	-/-	-/-	7-11	100%	92.9%	0.50	1.00	85.7%	100%	1.00	0.33	86.7%
Goel et al., 2011[24]	CS	15-50	30	Anterior	Maxilla/mandible	9	90.9%	100%	1.00	0.95	100%	90.9%	0.95	1.0	96.7%
Parvathy et al., 2014[21]	CS	-/-	20	Anterior	Maxilla/mandible	8-11	100%	100%	1.00	1.00	100%	100%	1.00	1.00	100%
Sandhu et al., 2015[27]	CS	15-50	16	Anterior	Maxilla/mandible	6-12	100%	100%	1.00	1.00	100%	100%	1.00	1.00	100%
Khambete et al., 2015[28]	CS	19-40	10	Anterior	Maxilla/mandible	8-11	100%	100%	1.00	1.00	100%	100%	1.00	1.00	100%

Sensitivity, specificity, PPV, NPV, and accuracy calculated from raw data if not reported by the authors.
-/-, Missing or not specified; *CS*, case series; *CR*, case report; *PG*, periapical granuloma; *PC*, periapical cyst; *PPV*, positive predictive value; *NPV*, negative predictive value.

In all studies except one,[21] the age range of the patients was presented. All studies except one[22] indicated the anatomic locations of the lesions: In almost all the studies, only anterior teeth were evaluated, with only one study including data on posterior teeth[23] and another study not specifying any difference.[22] Six studies reported information for both dental arches, and in one study[20] only two teeth in the maxilla were evaluated. Data were statistically analyzed to evaluate sensitivity, specificity, PPV, and NPV for either periapical cysts or periapical granulomas. Apical abscesses and combined lesions were also investigated, but numeric data were considered insufficient for a statistical analysis. Six studies showed 100% for sensitivity, specificity, PPV, and NPV. Three studies showed lower efficacy. The study from Goel et al.[24] showed the lowest sensitivity and NPV and the studies from Raghav et al.[25] and Prince et al.[22] showed the lowest specificity in the diagnosis of periapical granuloma. The study from Prince et al.[22] exhibited the lowest PPV. In the diagnosis of periapical cysts, the lowest sensitivity value and NPV were reported by Prince[22] and the lowest specificity and PPV values by Goel.[24] For all studies, it was possible to calculate the accuracy, being 100% for six studies, with the lowest score of 86.7% presented by Prince.[22]

Descriptive data from reports of clinical cases of lesions with different characteristics are shown in Table II. Gender, age, anatomic location of the lesion, and sample size were reported in all the studies. A positive correlation between ultrasonography imaging and histologic diagnosis was reported in all studies. Only three studies reported data on the frequency of the probe.

Clinical trials which reported a series of cases where the potential of ultrasonography was investigated in the evaluation of lesions of the jaws in general (not selectively of endodontic origin) are shown in Table III. If the studies presented findings on both soft tissue and intraosseous lesions, only the features of the bone lesions were extracted. Osteomyelitis, nonodontogenic cysts, odontogenic calcifying cyst, residual cyst, ossifying fibroma, neuroblastoma, vascular malformation, Ewing sarcoma, and osteosarcoma were positively identified in one study. Periapical granuloma and central giant cell granuloma were positively identified in two studies. Abscess, dentigerous cyst, and keratocystic odontogenic tumor were positively identified in three studies. Periapical cyst and ameloblastoma were identified in four studies.

A summary of the ultrasonographic features of lesions in the jaws identified in the papers under review is shown in Table IV. These "common features" are proposed as the gold standard for future ultrasonography

Table II. Data summary of case reports of lesions of different nature (in chronologic order)

Author	*Gender*	*Age*	*Sample (n)*	*Location*	*Frequency MHz*	*Histology*	*Ultrasonography features*	*Correlation between ultrasonography and histology*
Okita et al., 1991[32]	M	20	1	Maxilla	-/-	Dentigerous cyst	Homogeneous cystic area	+
Ng et al., 2001[41]	M	23	1	Mandible	5-10	Osteosarcoma	Bone thinning and erosion of buccal cortex with 90° bone spicules (sun−burst appearance) associated with a soft tissue mass	+
Roic et al., 2003[34]	M	9, 11	2	Mandible	-/-	1 ABC 1 CGCG	ABC: Complex mass containing anechoic areas separated by fibrous tissue CGCG: Expansive soft-tissue mass	+
Bialek et al., 2004[33]	F	17, 60	2	Mandible	7.5	Cyst -/-	Mandible body widened Anechoic polycyclic mass distorting the shape of mandible, posterior enhancement and no blood vessels	+
Oliveira et al., 2009[42]	F	11	1	Mandible	-/-	Chondrosarcoma	Solid content	+
Friedrich et al., 2010[35]	M	23	1	Mandible	-/-	AOT	Fluid-filled bony cavity with tooth crown inside	+
Bronoosh et al., 2011[36]	M	23	4	Maxilla/ mandible	-/-	3 KCOT parakeratinized 1 KCOT orthokeratinized	3 Well-defined hypoechoic with no CPD signal 1 well defined with mixed echogenicity no CPD signal	+
Sumer et al., 2012[37]	F	30	1	Mandible	-/-	KCOT	Ill-defined dens cystic content with no internal vascularity	+
Sheikh et al., 2012[39]	M	20	1	Mandible	9.6	TBC osteomyelitis	Generally described as an irregular suppurative lesion with erosion of bone	+
Shailaja et al., 2012[40]	F	18	1	Mandible	-/-	AVM	Large draining veins and fistulous communications with arteriovenous shunts with mixing blood and high velocity of 320 cm/s	+
Shaidi et al., 2012[43]	M	24	1	Mandible	5-13	Mesenchymal chondrosarcoma	Soft tissue mass, expansion and erosion of the buccal cortex, new bone formation in perpendicular spicules with sun-ray appearance.	+

-/- data absent or not specified.
+ positive correlation according to the author.
− negative correlation according with the author.
ABC, Aneurismal bone cyst; *AOT*, adenomatoid odontogenic tumor; *AVM*, arteriovenous malformation; *CGCG*, central giant cell granuloma; *KCOT*, keratocystic odontogenic tumor; *TBC*, tuberculosis.

investigations. Not all lesions were supported by sufficient descriptive data, and only the lesions with a qualitative description of the ultrasonographic findings are shown in the table.

DISCUSSION

The results of our systematic review of the literature have shown promising outcomes with regard to intraosseous lesions of the jaws. Information in the studies regarding soft tissue was not included in the review.

The endodontic studies that investigated apical periodontitis exhibited high diagnostic scores through statistic elaboration in terms of sensitivity, specificity, PPV, NPV, and accuracy. These studies shared a common study design and presented either raw data or statistical indices. In all these studies, ultrasonographic examination was able to detect apical periodontitis in all the cases. The pilot study by Cotti et al.[3] proposed the original protocol and laid the groundwork for following studies. A cystic lesion was described as a "transonic or hypoechoic lesion with well-defined boundaries and no evidence of internal vascularity on colour power-Doppler (CPD)," whereas an apical granuloma as a "poorly defined echogenic area with a rich vascular

Table III. Presentation of data summary from case series of lesions with different nature (in chronologic order)

	Lesions of various nature in case series																	
Authors	*Abscess*	*Osteomyelitis*	*Periapical granuloma*	*Non odontogenic cyst*	*Odontogenic cyst*	*Odontogenic calcifying cyst*	*Dentigerous cyst*	*Periapical cyst*	*Residual cyst*	*Kerartocystic odontogenic tumour*	*Ameloblastoma*	*Ossifying fibroma*	*Central giant cell granuloma*	*Neuroblastoma*	*Vascular malformation*	*Ewing's sarcoma*	*Osteosarcoma*	*No. lesions identified*
Dib et al., 1996 ([44])	-/-	-/-	-/-	-/-	-/-	2(m)	9(c)	8(c)	-/-	7(k)	12(s) 11(m)	9(s)	2(s)	1(s)	-/-	-/-	-/-	61/65
Kohout et al., 1998[45]	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	7(v)	-/-	-/-	7/7
Sumer et al., 2009[46]	-/-	-/-	4(s)	-/-	-/-	-/-	2(m)	6(c+m)	3(c+m)	3(k)	-/-	-/-	-/-	-/-	-/-	-/-	-/-	18/19
Lu et al., 2009[38]	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	12(m) 4(c) 3(s)	-/-	-/-	-/-	-/-	-/-	-/-	19/19
Bagewadi et al., 2010[47]	1(a)	-/-	-/-	-/-	9(c)	-/-	-/-	-/-	-/-	-/-	3(b)	-/-	-/-	-/-	-/-	-/-	1(mL)	14/17
Chandak et al., 2011[48]	6(i)	-/-	-/-	2(c)	2(c)	-/-	-/-	4(c)	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	14/14
Pallagatti et al., 2012[49]	5(i)	1(i)	1(i)	-/-	12(c)	-/-	-/-	-/-	-/-	-/-	3(b)	-/-	1(b)	-/-	-/-	1(mL)	-/-	24/25

-/-: Absent or not-specified.

a, Abscess content; *b*, benign tumor; *i*, inflammatory content; *c*, simple cystic content; *k*, complex cyst or cyst containing keratin; *m*, mixed or semisolid content; *mL*, malignant tumor; *s*, solid content; *v*, vascular malformation.

Table IV. Presentation of the common features of lesions reported in literature as described with ultrasonography

Lesion	*No. of lesions*	*US features*	*Studies*
Suppurative lesions (abscess and infected cyst)	(n = 13)	Often described as anechoic to hypoechoic area that can be homogenous or with both anechoic and hypoechoic areas, irregular shape and boundaries and no evidence of internal vascularity on CPD	28, 47-49
Osteomyelitis*	(n = 1)	Generally described as an irregular suppurative lesion with erosion of bone	39
Periapical granuloma	(n = 51)	Often described a frankly echogenic area (which can contain both hypoechoic and hyperechoic areas) with poorly defined boundaries and a rich vascular supply on CPD	20-28, 46
Combined lesion	(n = 5)	Described as mixed lesions with echogenic and anechoic areas and showing vascularity in some areas (most in periphery) on CPD	23, 22
Intraosseus cyst no otherwise specified	(n = 23)	Commonly described as an anechoic area, with homogenous internal pattern, round shape, very clear boundaries and enhanced posterior echoes	47, 48
Odontogenic calcifying cyst	(n = 2)	Described as a mixed lesion showing both solid and cystic areas	44
Dentigerous cyst	(n = 12)	Some described as anechoic area with focal hyperechogenicity attributed by some authors to the crown of a tooth	44, 46, 47, 49
Periapical cyst	(n = 89)	Often described as anechoic to hypoechoic area with well-defined reinforced boundaries and no sign of internal vascularity on CPD	20-26, 28, 44, 46, 47-49
Residual cyst	(n = 3)	Some described it as an anechoic, or anechoic and hypoechoic area	46
Keratocystic odontogenic tumor	(n = 17)	Often described as an hypoechoic area with a dens content and no evidence of internal vascularity on CPD	44, 46, 47, 49
Ossifying fibroma	(n = 9)	Described as a solid echogenic mass	44
Central giant cell granuloma	(n = 4)	Described as a solid echogenic mass	44, 49
Neuroblastoma	(n = 1)	Described as a solid echogenic mass	44
Ameloblastoma	(n = 48)	Described a s an heterogenic lesion with hyperechoic, hypoechoic and anechoic areas, variable boundaries and in some cases internal compartments and from minimal to abundant color Doppler signal	38,44, 47, 49
Arteriovenous malformations *	(n = 1)	Described as a lesion containing arteriovenous shunts with mixing blood and high velocity flow on CPD	40
Ewing's sarcoma	(n = 1)	Described as a complex echo texture, heterogeneous internal echo pattern and irregular boundaries	49
Osteosarcoma	(n = 1)	Described as a hyperechoic to anechoic lesion with heterogeneous internal echoes with irregular shape and boundaries with thinning, expansion and sun ray appearance of the surrounding bone	47
Chondrosarcoma*	(n = 1)	Described as an echogenic solid area with erosion and expansion of the cortex, new bone formation in hyperechoic perpendicular spicules with sun-ray appearance.	43

CPD, Color power Doppler.
*These lesions are described only in the case reports (CR) presented in Table II.

supply on CPD." The original pilot study did not include the so-called mixed lesions (i.e., lesions with a mixed ecographic pattern between a cyst and a granuloma); nevertheless, the follow-on study[23] identified two apical lesions with a more complex ultrasonographic pattern (in gray-scale and CPD) than the ultrasonographic extremes of the apical cyst and granuloma. They diagnosed a periapical cystic lesion containing a dense material within the lumen, which histopathology proved to be a periapical cyst with mucus-secreting goblet cells, and a combined lesion resulted in a granuloma-containing a cyst. The same authors confirmed these findings in a further study.[20] In the study by Gundappa et al.,[26] mixed lesions were integrated in the ultrasonographic protocol and defined as lesions with either hypoechoic or anechoic areas with vascularity in some areas. Using this protocol, four apical cysts, seven apical granulomas, and a combined lesion were detected and diagnosed through ultrasonography and confirmed with histopathology. Three lesions showed mixed echogenicity with internal scattering echoes and no evidence of internal vascularity, suggestive of a suppurative process; these lesions were diagnosed and confirmed as infected cysts. In two cases in the same study, ultrasonography was able to detect and trace sinus tracts. Further studies added new data and confirmed the previous findings, with 100% score in sensitivity, specificity, and accuracy and a score of 1.00 in PPV and NPV compared with the gold standard.[21,22,24,25,27,28]

Three studies did not reach the 100% score; however, they showed higher accuracy than other studies employing cone beam computed tomography in the differential diagnosis of apical periodontitis, in which patients are exposed to ionizing radiations.[29] In the study by Goel et al.,[24] a lesion was misdiagnosed, but the author explained that the possible reason for the misdiagnosis could have been an error that occurred during the processing of the lesion, either during surgery or the histologic preparation of the sample. Raghav et al.[25] reported a high accuracy score of 95.2%; however, they did not explain the misdiagnosed case. The study from Prince et al.[22] showed the lowest accuracy (86.7%), but a reason for this value could be found in the paper itself: A lesion appearing as solid in gray scale was diagnosed as a granuloma, even though there was lack of internal vascularity on CPD, which is highly suggestive of an apical cyst. The solid appearance can be explained by the possibility that occasionally cysts containing debris, cholesterol clefts, and keratin might appear as echogenic in gray scale. In endodontic lesions, differential diagnosis between a cystic lesion and a granuloma can be of assistance when deciding between root canal and surgical management; however, ultrasonography is unable to discriminate between true cysts and pocket cysts, as described by Nair.[30,31]

The evidence available with regard to lesions of the jaws in general was not as robust as for apical periodontitis. Eleven case reports and seven case series were included in the systematic review for the latter. Since it was not possible to carry out a numeric analysis of the studies, a correlation between the diagnoses made on the basis of ultrasonography and the definitive histopathologic report was examined. All papers analyzed reported a positive correlation and positive clinical value of ultrasonography in detecting lesions in the bone.

When examining case reports, cystic lesions were detected and diagnosed using ultrasonography in two studies,[32,33] whereas in one of the studies[33] the cystic lesions were incidental findings, suggesting the high sensitivity of this technique and that the technique can occasionally be the first tool that reveals unsuspected lesions in the jaws. Further examples of incidental findings are an aneurysmal bone cyst and a central giant cell granuloma discovered while investigating preauricolar swellings in children, as reported by Roic et al.[34] One case showed an adenomatoid odontogenic tumor associated with an impacted lower canine, and the authors suggested that during ultrasonography, the tip of the crown was visualized as being surrounded by a fluid-filled cavity. However, in this study CPD was not applied to assess the vascularity of the lesion.[35] Two studies investigating keratocystic odontogenic tumors ([KCOT] formerly odontogenic keratocyst) had inconsistent findings regarding the boundaries of this lesion; in one of them ill-defined borders were reported, whereas well-defined contours were described in the other study.[36,37] We can speculate that KCOT contours can be different because of the increased or reduced aggressive behaviors of the lesion, as suggested by Lu et al.[38] A study reported a case of mandibular osteomyelitis resulting from tuberculosis infection, in which chest radiography, the Mantoux test, and staining of acid-fast bacilli were negative, and the echographic finding was highly suggestive of an intraosseous suppurative process.[39]

Radiographic appearance of vascular malformations can be confused with other lesions, with subsequent intraoperative surgical risks. One of the studies detected an unusual vascular condition and measured high-flow speed with CPD to differentiate lymphatic, venous, and arteriovenous malformations with success.[40] Three case reports[41-43] reported malignancies, which were then confirmed by histopathology. In two of them,[41,43] the signs of malignancy, such as bone destruction and pathologic calcifications of bone spicules with the pathognomonic sun-ray appearance, were detected by ultrasonography.

Furthermore, the present systematic review included seven case series investigating lesions of various natures in the jaws.[38,44-49] Unfortunately, the heterogeneity of these studies impeded a quantitative summary of the findings. A pilot study by Dib et al.[44] concluded that the use of ultrasonography should be recommended as a complementary routine for the diagnosis of lesions in the jaws and as guide for biopsies in mixed lesions. Seven of 72 lesions in this study could not be detected with ultrasonography because some small lesions may be surrounded by a thick cortical bone plate acting as a barrier to ultrasound waves. Ultrasonographically inconclusive cases included lesions containing mineralized tissue, such as ossifying fibroma or dentigerous cysts, which could also act as a barrier to ultrasound waves passing through the tissue. Twenty-eight lesions considered hyperechoic (but less echogenic if compared with the surrounding bone) were diagnosed as solid tumors, and 24 of them were confirmed histologically as ossifying fibroma, ameloblastoma, central giant cell granuloma, or neuroblastoma. Seventeen transonic or anechoic lesions were diagnosed as cysts with liquid content, and all of them were confirmed histologically as periapical cyst or dentigerous cysts. The 13 mixed lesions diagnosed as tumors presenting either solid or cystic contents were all histologically described as ameloblastomas or odontogenic calcifying cysts. Seven lesions with dense liquid content were diagnosed and confirmed as KCOTs. In this study, 61 of the ultrasonographic diagnosis matched the histologic report (93.8% of accuracy). In four cases, the ultrasonography results did not match those of histology: Two lesions considered solid were diagnosed histologically as infected cysts, probably as a result of the presence of a thick and inflamed capsule and a sinus tract. The remaining two lesions, finally confirmed to be KCOTs, mimicked a solid lesion, likely because of the internal keratin. The use of CPD in the latter four cases would likely have been useful in the differential diagnosis by revealing the lack of internal vascularity in the cystic lesions.

A subsequent similar study added the use of CPD to the gray-scale imaging evaluation and divided the lesions into four categories. In this study, three of 22 lesions could not be detected and were therefore defined as "inconclusive," with 18 of 19 lesions detected were confirmed histologically (94.7% accuracy). Five anechoic lesions, with or without limited internal echoes and no evidence of internal vascularity, were histologically confirmed as cysts. Three lesions containing dense internal echoes and no evidence of internal vascularity were diagnosed and confirmed as KCOTs.[46] An important fact of this study is that six cystic lesions were described as semisolid in the gray scale and showed no evidence of internal vascularity. Five lesions that showed solid appearance and internal vascularity on CPD were diagnosed as apical granulomas, although histology reported one of them as being a cyst. The authors explained that the apical cyst was characterized by a thick capsule presenting histologic signs of chronic and active inflammation; however, according to Cotti[23] and Gundappa,[26] this lesion should have been diagnosed as a combined lesion instead. We should emphasize that only few studies specified in detail the procedure for processing biopsy samples, considering that an accurate diagnosis of apical cyst is possible only through serial sectioning or step serial sectioning of the lesions removed in toto.[30,31]

Three studies evaluated head and neck swellings presenting in hard or soft tissues, with lesions classified according to their shape, contours, echo intensity, internal architecture, and vascularity assessed through CPD.[47-49] In these studies, ultrasonography was used to diagnose abscesses, with diagnosis confirmed histologically in 12 out of 15 lesions, whereas 29 cystic lesions were correctly diagnosed out of 30 lesions.[47-49] Other types of bone lesions found included malignancies,[47,49] benign odontogenic tumors, which were histologically diagnosed as ameloblastomas,[47,49] and a central giant cell granuloma.[49]

Ultrasonography has several advantages over conventional radiography in the study of unilocular lesions of the jaws, where similar radiographic pattern can affect the differential diagnosis. Chandak et al.[48] found relatively high sensitivity and specificity when comparing ultrasonographic findings with those of histology, with a statistically significant association in the differential diagnosis of head and neck lesions overall. This was confirmed by two other studies, which found a significant association between ultrasonographic examination and histologic diagnosis with values of contingency.[47,49]

Similarly, ultrasonography has been used to detect and diagnose lesions and to establish resection margins intraoperatively, with the application of CPD being discussed in a retrospective case series regarding vascular malformations.[45]

The potential of ultrasonography in detecting the risk of aggressive lesion behavior and to evaluate the solid or cystic nature ameloblastomatous lesions has been explored. Ultrasonography was able to detect the lesions in all 19 cases evaluated, with 100% sensitivity and 94% specificity in predicting the active proliferation of the tumor using color Doppler. Lesions with active proliferation showed an abundant flow signal. Also, the boundaries were evaluated to predict tumor proliferation, with lesions with unclear or partially unclear contours revealing an aggressive behavior according to histopathology. This particular assessment presented with a sensitivity of 100% and a specificity of 88%.[38] These findings have clinical importance, since only aggressive lesions should be treated with osseous resection.

Although the results of this review seem to be very promising, it should be kept in mind that it is not possible that every clinical case or every element of a study is reported and that studies involving a diagnostic technique are likely to be affected by publication bias. It has been demonstrated that studies presenting positive results are more likely to be published and are published in journals with a higher impact factor than in those exhibiting null results.[50,51]

CONCLUSIONS

The results of this systematic review demonstrate the value of ultrasonography for the evaluation of the nature of intraosseous lesions in the jaws, in particular for the differential diagnosis between apical cysts and granulomas. Therefore, ultrasonography should be considered an additional imaging technique in routine dentistry and maxillofacial surgery when a diagnostic problem arises.

REFERENCES

1. American Institute of Ultrasound in Medicine. The AIUM celebrates 50 years of excellence. Available at: http://www.aium.org/aboutUs/history/articles/jum1.pdf. Accessed October 9, 2015.

2. Goldstein A. Overview of the physics of US. *Radiographics*. 1993;13:701-704.
3. Cotti E, Campisi G, Garau V, Puddu G. A new technique for the study of periapical bone lesions: ultrasound real time imaging. *Int Endod J*. 2002;35:148-152.
4. Cotti E, Campisi G. Advanced radiographic techniques for the detection of lesions in bone. *Endod Top*. 2004;7:52-72.
5. Girish G, Jacobson JA. Ultrasound. In: Weissman BN, ed. *Imaging of Arthritis and Metabolic Bone Disease*. Philadelphia, PA: Saunders; 2009:89-103.
6. Moore CL, Copel JA. Point-of-care ultrasonography. *N Engl J Med*. 2011;364:749-757.
7. Martin K. Basic equipment, components and image production. In: Allan PL, Baxter GM, Weston M, eds. *Clinical Ultrasound*. 3rd ed. St. Louis, MO: Churchill Livingstone; 2011:16-30.
8. McDicken WN, Anderson T. The difference between colour Doppler velocity imaging and power Doppler imaging. *Eur J Echocardiogr*. 2002;3:240-244.
9. Brown BSJ. How safe is ultrasonography? *Can Med Assoc*. 1984;131:307-311.
10. Carstensen EL. Acoustic cavitation and the safety of diagnostic ultrasound. *Ultrasound Med Biol*. 1987;13:597-606.
11. Miller DL. Update on safety of diagnostic ultrasonography. *J Clin Ultrasound*. 1991;19:531-540.
12. Nyborg WL. Scientifically based safety criteria for ultrasonography. *J Ultrasound Med*. 1992;11:425-432.
13. Duck FA. Recent epidemiology papers: European committee for medical ultrasound safety. *Ultraschall Med*. 2001;13:227-231.
14. Salvesen KA. Epidemiology of diagnostic ultrasound exposure during pregnancy. *Ultraschall Med*. 2002;15:165-171.
15. Marotti J, Heger S, Tinschert J, et al. Recent advances of ultrasound imaging in dentistry—a review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2013;115:819-832.
16. Shah N, Bansal N, Logani A. Recent advances in imaging technologies in dentistry. *World J Radiol*. 2014;28:794-807.
17. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009;151:264-269.
18. Coroneos CJ, Ignacy TA, Thoma A. Designing and reporting case series in plastic surgery. *Plast Reconstr Surg*. 2011;128: 361-368.
19. Gagnier JJ, Kienle G, Altman DG, et al. The CARE guidelines: consensus-based clinical case reporting guideline development. *Headache*. 2013;53:1541-1547.
20. Cotti E, Simbola V, Dettori C, Campisi G. Echographic evaluation of bone lesions of endodontic origin: report of two cases in the same patient. *J Endod*. 2006;32:901-905.
21. Parvathy V, Kumar R, James EP, George S. Ultrasound imaging versus conventional histopathology in diagnosis of periapical lesions of endodontic origin: a comparative evaluation. *Indian J Dent Res*. 2014;25:54-57.
22. Prince NC, Annapurna CS, Sivaraj S, Ali IM. Ultrasound imaging in the diagnosis of periapical lesions. *J Pharm Bioallied Sci*. 2012;4:183-185.
23. Cotti E, Campisi G, Ambu R, Dettori C. Ultrasound real-time imaging in the differential diagnosis of periapical lesions. *Int Endod J*. 2003;36:556-563.
24. Goel S, Nagendrareddy SG, Raju MS, et al. Ultrasonography with color Doppler and power Doppler in the diagnosis of periapical lesions. *Indian J Radiol Imaging*. 2011;21:279-283.
25. Raghav N, Reddy SS, Giridhar AG, et al. Comparison of the efficacy of conventional radiography, digital radiography and ultrasound in diagnosis of periapical lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;110:379-385.
26. Gundappa M, Ng SY, Whaites EJ. Comparison of ultrasound, digital and conventional radiography in differentiating periapical lesions. *Dentomaxillofac Radiol*. 2006;35:326-333.
27. Sandhu SS, Singh S, Arora S, Sandhu AK, Dhingra R. Comparative evaluation of advanced and conventional diagnostic aids for endodontic management of periapical lesions: an in vivo study. *J Clin Diagn Res*. 2015;9:1-4.
28. Khambete N, Kumar R. Ultrasound in differential diagnosis of periapical radiolucencies: a radiohistophatological study. *J Conserv Dent*. 2015;18:39-43.
29. Guo J, Simon JH, Sedghizadeh P, Soliman ON, Chapman T, Enciso R. Evaluation of the reliability and accuracy of using cone-beam computed tomography for diagnosing periapical cysts from granulomas. *J Endod*. 2013;39:1485-1490.
30. Nair PNR, Pajarola G, Schroeder HE. Types and incidence of human periapical lesions obtained with extracted teeth. *Oral Surg Oral Med Oral Pathol*. 1996;81:93-102.
31. Nair PNR. New perspectives on radicular cysts: do they heal? *Int Endod J*. 1998;31:155-160.
32. Okita W, Ichimura K, Linuma T. Dentigerous cyst of the maxilla and its image diagnosis. *Rhinology*. 1991;29:307-314.
33. Bialek EJ, Jakubowoski W, Zajkowoski P. Ultrasonography as the method of incidental detection of mandible lesions. *Acta Otorhinolaryngol Belg*. 2004;58:157-159.
34. Roic G, Posaric V, Marusic A, Boric I, Vlahovic T, Vrlicek K. Unexpected diagnosis for preauricular swelling—two case reports. *Radiol Oncol*. 2003;37:155-159.
35. Friedrich RE, Zustin J, Scheuer H. Adenomatoid odontogenic tumour of the mandible. *Anticancer Res*. 2010;30:1787-1792.
36. Bronoosh P, Shakibafar A, Houshyar M, Nafarzade S. Imaging findings in a case of Gorlin-Gotz syndrome: a survey using advanced modalities. *Imaging Sci Dent*. 2011;41:171-175.
37. Sumer AP, Sumer M. Celenk, P, Danaci M, Gunhan O. Keratocystic odontogenic tumor: a case report with CT and ultrasonography findings. *Imaging Sci Dent*. 2012;42:61-64.
38. Lu L, Yang J, Liu J-B, Yu Q, Xu Q. Ultrasonographic evaluation of mandibular ameloblastoma: a preliminary observation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;108:32-38.
39. Sheikh S, Pallagatti S, Gupta D, Mittal A. Tuberculous osteomyelitis of mandibular condyle: a diagnostic dilemma. *Dentomaxillofac Radiol*. 2012;41:169-174.
40. Shailaja SR, Manika Manjula M, Kumar LV. Arteriovenous malformation of the mandible and parotid gland. *Dentomaxillofac Radiol*. 2012;41:609-614.
41. Ng SY, Songra A, Ali N, Carter JLB. Ultrasound features of osteosarcoma of the mandible—a first report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2001;92:582-586.
42. Oliveira RC, Marques KDS, Mendoca AR, et al. Chondrosarcoma of the temporomandibular joint: a case report in a child. *J Orofac Pain*. 2009;23:275-281.
43. Shahidi S, Shakibafard A, Zamiri B, et al. Ultrasonographic findings of mesenchymal chondrosarcoma of the mandible: report of a case. *Imaging Sci Dent*. 2012;42:115-119.
44. Dib LL, Curi MM, Chammas MC, Pinto DC, Torloni H. Ultrasonography evaluation of bone lesions of the jaw. *Oral Surg Oral Med Oral Pathol*. 1996;82:351-357.
45. Kohout MP, Hansen M, Pribaz JJ, Mulliken JB. Arteriovenous malformations of head and neck; natural history and management. *Plast Reconstr Surg*. 1998;102:643-654.
46. Sumer AP, Danaci M, Sandikci EO, Sumer M, Celenk P. Ultrasonography and Doppler ultrasonography in the evaluation of intraosseus lesions of the jaws. *Dentomaxillofac Radiol*. 2009;38:23-27.
47. Bagewadi S, Mahima VG, Patil K. Ultrasonography of swellings in orofacial region. *J Indian Acad Oral Med Radiol*. 2010;22:18-26.

48. Chandak R, Degwekar S, Bhowte RR, et al. An evaluation of ultrasonography in the diagnosis of head and neck swellings. *Dentomaxillofac Radiol*. 2011;40:213-221.
49. Pallagatti S, Sheikh S, Puri N, Mittal A, Singh B. To evaluate efficacy of ultrasonography compared to clinical diagnosis radiography and histopathological findings in the diagnosis of maxillofacial swellings. *Eur J Radiol*. 2012;81:1821-1827.
50. Easterbrook PJ, Berlin JA, Gopalan R, Matthews DR. Publication bias in clinical research. *Lancet*. 1991;337:867-872.
51. Dickersin K. The existence of publication bias and risk factors for its occurrence. *JAMA*. 1990;263:1385-1389.

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Chapter 4

Ultrasound examination with color power Doppler to assess the early response of apical periodontitis to the endodontic treatment

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Ultrasound examination with color power Doppler to assess the early response of apical periodontitis to the endodontic treatment

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Abstract

Objectives The purpose of this study was to assess the possibility to detect early vascular changes in apical periodontitis (AP) using ultrasound examination with color power Doppler (US-CPD) and to establish a correlation between the early response of AP to treatment and its potential healing.

Materials and methods Twenty-one apical lesions were visualized with US-CPD before endodontic treatment, 1 week after the first access to endodontic system and 4 weeks after root canal obturation. A differential diagnosis between cystic lesions (CLs) and granulomatous lesions (GLs) was attempted. The vascular modifications were then correlated with long-term radiographic follow-up using Fisher's exact test.

Results The decrease or disappearance of vascular flow observed in AP 4 weeks after root canal obturation was significantly related to a healing trend ($p = 0.0206$) of the lesions. Combining the data with preoperative US-CPD diagnosis showed a reproducibility for GLs only ($p = 0.0022$).

Conclusions This study showed the possibility to detect early vascular changes in AP using US-CPD, correlating them with a healing trend after endodontic treatment. Future investigations should be conducted and more attention should be dedicated to the potential of this alternative and biologically safe imaging technique.

Clinical relevance US-CPD in endodontics may be a helpful tool to identify healing processes after endodontic treatment and to understand the behavior of different forms of AP.

Keywords Apical periodontitis · Ultrasound examination · Color power Doppler · Healing

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Introduction

Ultrasound examination (echography) has been used in medicine for more than 50 years and is considered a reliable, cost-effective, and safe imaging technique [1–3]. When ultrasound (US) waves produced by a probe encounter biological tissues with different mechanical and acoustic properties, part of the energy is transmitted through the tissues, while part of it is reflected back to the transducer (i.e., an echo). Ultrasound waves are then converted into electric current and gray-scale images on a screen. The images are renewed many times per second to produce a moving image on the screen [1, 4]. US examination is enhanced with Doppler systems, which use the Doppler effect to obtain a real-time two-dimensional image of vascularity of the examined tissue as a color overlay on a B-mode image. Two types of Doppler imaging are considered here: color Doppler (CD) and power Doppler (PD). CD represents the mean velocity of blood cells at a given time on a color scale, where red and blue are commonly used to indicate flow toward and away from the transducer, respectively. Low velocities are displayed as dark hues, whereas high velocities are displayed as lighter shades of color. PD is a measure of the amount of moving blood cells in the sample volume, and it has a higher sensitivity than CD for depicting flow in small vessels [5, 6].

Dentistry is strongly dependent on imaging systems, and US examination has been used previously in endodontics with success for facilitating the differential diagnosis of apical periodontitis (AP) [7–10]. A recent systematic review concluded that ultrasounds with color power Doppler (US-CPD) had high scores of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy in the differential diagnosis of AP when compared to the histopathologic analysis [11]. Few studies have used US-CPD to evaluate long-term changes in vascularity in periapical tissues following non-surgical [12, 13] and surgical [14] endodontic treatment of AP.

The results from a pilot study [15] have demonstrated that in cases of AP, US-CPD can be used to identify early changes in vascularity of the lesions following endodontic treatment. The purpose of this preliminary report was to evaluate the effectiveness of US-CPD in detecting the presence of early changes in the vascular supply of periapical tissues and predict the outcome of endodontic treatment. The rationale was that variations in vascularity within and/or around AP following endodontic treatment would represent changes in the inflammatory response of the periapical tissues and could be interpreted as a trend for the healing response.

Materials and methods

Nineteen healthy patients with a non-contributory medical history (8 males and 11 females, with an average age of 38.9 years) and a total of 21 apical lesions were selected among the patients who had been referred to an endodontic office for the treatment of AP in the last 7 years. The inclusion criteria were as follows: (1) healthy patients (without systemic conditions), (2) age between 20 and 70 years, (3) taking no medication, (4) the presence of at least one tooth with AP, and (5) willingness to participate in the study. Patients who agreed to participate in the study were asked to sign an informed consent. The exclusion criteria were the following: (1) patients with systemic diseases, (2) patients who did not fit in the age category, (3) patients taking medications, (4) teeth with fractured roots or periodontal disease, and (5) patients who did not agree to participate in the study. A total of 26 teeth were included in the protocol. All the patients selected were diagnosed with chronic AP in one or more teeth, at the first visit, based on radiographic features of AP and negative responses to both the CO_2 pulp sensitivity test and the electric pulp test. Some of the cases exhibited a sinus tract [16–18]. Periodontal status was assessed following the Ramfjord periodontal index [19] using a calibrated periodontal probe on the affected teeth to exclude the presence of vertical root fractures or periodontal disease. The treatment plan involved primary orthograde root canal treatment (PRCT) on 10 teeth, orthograde secondary root canal treatment (SRCT) on 12 teeth, and surgical endodontic treatment of 4 teeth.

Clinical protocol

The clinical protocol was designed as follows:

a. PRCT and SRCT on 22 teeth to be completed in two appointments
b. Radiographic assessment: (1) preoperative, (2) intraoperative, (3) postoperative; (4) 6 months postoperative, (5) 1 year after treatment, and (6) once a year following the 1-year follow-up
c. Three US-CPD examinations: (1) before treatment (preoperative); (2) 1 week after root canal debriding, cleansing, and disinfecting (interoperative); and (3) 4 weeks after completion of the root canal treatment (postoperative)

Radiographs

Preoperative, intraoperative, and postoperative periapical radiographs were obtained for each patient using either traditional or digital devices for the parallel technique. Traditional radiographs were acquired using F-speed films (Insight, Eastman-Kodak Co., Rochester, NY, USA). Digital radiographs were acquired with a CMOS system (Snapshot, Instrumentarium Dental, and Tuusula, Finland) and processed with software to adjust the brightness and the contrast. The radiographs were taken with the vertical, long axis of the affected tooth fixed perpendicularly to the central ray and parallel to the sensor and film at a 40-cm focal spot-to-object distance. The geometric alignment between the sensor or film and the tooth was standardized using a fixing device. The dental X-ray unit (Kodak 2200, Carestream, and Rochester, NY, USA) operated at 70 kVp and 10 mA. The exposure time was 0.08 ms for the digital images and 0.320 ms for the F-speed film. Cone beam computed tomography (CBCT) was not performed in all the patients due to the absence of insurance benefits and the potential risks of the examination outweighing the value of such an examination in these cases [20].

Ultrasound examination

US examination was performed by an expert sonologist using either a Siemens Elegra (Siemens, Erlangen, Germany) or a Toshiba Aplio XG (Toshiba Medical Systems, Crawley, UK) apparatus with regular and small-size, linear, high-definition, multi-frequency US probes. The first and second probes were used at a frequency of 7 to 9 and 8 to 12 MHz, respectively. After the application of an echographic gel, the probe was placed both extra-orally on the skin of the upper and lower jaw and intra-orally in the area of the alveolar mucosa, corresponding to the roots of the involved tooth. The position of the probe was changed several times to obtain an adequate number of scans to define the bony defect. The CPD was applied to the gray-scale images to assess the presence, amount, and features of the vascular supply of each lesion (e.g., intra-lesional and/or peri-lesional vascularity). A tentative differential diagnosis between granulomatous lesions (GLs) and cystic lesions (CLs) was made by a trained sonologist and an expert endodontist during the preoperative examination according to the criteria described in the literature (7). All patients underwent two more US-CPD examinations 1 week following root canal debriding, cleansing, and disinfecting (interoperative) and 4 weeks after completion of the root canal treatment (postoperative).

Orthograde PRCT or SRCT

In 17 patients and 22 teeth, PRCT or SRCT was performed in two visits. The procedure of the first appointment consisted of the administration of local anesthesia, isolation with a rubber dam, caries removal, and opening of the access cavity/ disassembly of the restoration; the working length of the root canals was determined with an apex locator (Root-ZX, J. Morita Corp., Osaka, Japan) and confirmed by periapical radiographs. The shaping of the root canal space was performed using manually operated instruments. K-Flex files (K-Flex Files, Kerr Corp., Orange, CA, USA) no. 08 to no. 20 were used for scouting and preflaring, and nickel titanium rotary instruments (EndoWave, J. Morita Corp., Osaka, Japan) were used for shaping and apical preparation. In retreatment cases, the previous root canal filling was removed using Gates Glidden drills no. 1–3 and hand files with the complement of a solvent (Endosolv E, Septodont, Paris, France). Copious irrigation was performed with 5% sodium hypochlorite during and after the use of instrumentation. Teeth were temporarily restored with Cavit (3 M ESPE, St. Paul, MN, USA), and the patients were scheduled for a second visit approximately 1 week later. An intraoperative US-CPD examination of each patient was performed before the second appointment to evaluate changes in the peripheral and/or internal vascularization of the lesion. At the second appointment, after re-accessing the tooth as previously described, the root canals were checked for apical preparation, rinsed with sodium hypochlorite 5%, alternated to 17% ethylene diamine tetra acetic acid (EDTA; Ogna Lab Srl, Muggiò, Milano, Italy), dried, and filled with gutta-percha cones and AH Plus sealer (Dentsply DeTrey, Konstanz, Germany) using the warm vertical compaction technique. Teeth were then restored with composite resin and adhesive systems. At the 4-week recall visit, the third and last US-CPD examination was performed.

Surgical protocol

The surgical protocol was designed as follows:

a. Surgical endodontic treatment was performed on two patients and four teeth
b. Radiographic assessment: (1) preoperative, (2) postoperative, (3) 6 months and 1 year after treatment, and (4) once a year following the first 1-year follow-up
c. Three US-CPD examinations: (1) before treatment (preoperative), (2) 1 week after surgical procedure (interoperative), and (3) 4 weeks after soft-tissue healing completion and suture removal (postoperative)

Endodontic surgery

On two patients and four teeth, endodontic surgery was performed according to the protocol by Kim and Kratchman [21]. After local anesthesia with 2% mepivacaine with 1:100,000 epinephrine, a periodontal flap was elevated. The diseased apical root ends were accessed through osteotomy using a surgical round bur with continuous water irrigation, and the apical lesions were curetted and fixed in an aqueous solution of 10% neutral formalin for routine histopathological analysis. Root-end resections of 3 mm were then performed with an angle as perpendicular as possible to the long axis of each tooth, and approximately 3-mm root-end cavities were prepared using ultrasonic tips and continuous irrigation. The root ends were filled using MTA (ProoRoot MTA, DENTSPLY Tulsa dental, Tulsa, OK, USA), and the flaps were repositioned and sutured. The second US-CPD examination was performed at the appointment for suture removal (1 week after surgery). The third US-CPD was performed after 4 weeks from suture removal and clinical examination.

Control cases

One of the cases was also used as negative control case. In this case, the US-CPD examination was repeated two more times, at 1 and 4 weeks following the first visit, before starting the endodontic treatment, to observe whether significant variations in the vascular supply of the lesion could occur without performing any treatment on the tooth.

Two surgical cases were selected as positive control cases to document the healing of the bone cavity after the complete removal of the lesion by endodontic surgery.

Recall visits

The recall visits were scheduled at 1 week, 4 weeks, 6 months, and 1 year. An accurate clinical examination was repeated, and either traditional or digitized radiographs were obtained. Then, follow-up visits and radiographs were scheduled once a year.

Statistical analysis

The data related to the vascularization of the lesions at the US-CPD were evaluated by two trained endodontists and an expert sonologist. A consensus was reached after complete agreement between the three observers, and in the case of disagreement, a consensus was obtained after a discussion. Vascular features of the lesions were studied overlapping consecutively CD and PD to the gray-scale image to evaluate absence or presence of vascularity and distribution of the vascularity (peripheral or internal).

All cases were analyzed by correlating the occurrence of increase, decrease, disappearance, and persistence of the vascularization, from the beginning of treatment to the 4-week follow-up and, finally, to the healing of AP.

AP was considered either healed or healing when complete radiographic healing/normal periodontal ligament space was observed or when a significant reduction in the lesion size was observed in combination with the complete absence of clinical signs and symptoms, in the cases that had a shorter recall time [22].

Data were analyzed using Fisher's exact test coherently with the sample size to appraise a statistical significance ($p < 0.05$) measuring the deviation from a null hypothesis (two-tailed p value). The results are reported in Table 1.

Results

The average long-term clinical and radiological follow-up time to validate a healing/healing trend was of 2.9 years. In the negative control case, three subsequent US-CPD examinations were performed before intervention and did not show any appreciable variation in the vascularity of the lesion studied (Fig. 1). The two cases scheduled for endodontic surgery were diagnosed preoperatively as CLs, with the US-CPD showing a hypoechoic lesion with the absence of internal and peripheral vascularity. Surgical specimens were processed for histopathological analysis, and the diagnosis of CL was confirmed. One week after the removal of both lesions, the healing bone cavities showed a peripheral vascularity with US-CPD, which was still present at 4 weeks but with a reduction of the Doppler signal (Fig. 2). At the preoperative US-CPD analysis, 15 lesions were diagnosed as GLs, showing an internal Doppler signal within an echogenic content, whereas four lesions were classified as CLs. In three of the CLs, the US-CPD showed no evidence of vascularity around and within the hypoechoic image, whereas in one lesion, there was the presence of peripheral vascularity.

Healing of AP and US-CPD features at 1 week

At the interoperative examination with US-CPD, seven cases demonstrated a decrease (two cases) or disappearance (five cases) of the vascular signal within the lesion, and a healing of the periapical lesion was observed clinically and radiologically on follow-up. In 11 cases, no significant modifications in the intensity of the vascular signal within the lesion were identified; eight of these cases later showed radiographic healing, whereas three cases did not. One case showed an increase in vascularity at the 1-week US-CPD, and it healed. The modifications of the Doppler signal of the periapical lesions at 1 week and the radiographic healing of the same lesions showed no statistical significance ($p = 0.2632$).

Healing of AP and US-CPD features at 4 weeks

In 14 cases, the US-CPD postoperative examination showed decrease (six cases) or disappearance (eight cases) of the vascular signal and healing was observed for all the cases after the clinical and radiological follow-up. In five cases, the Doppler signal did not show any reduction after 4 weeks from the end of PRCT or SRCT: two of these cases healed, whereas three failed. The correlation between the reduction/disappearance of the Doppler signal and the healing of the lesion was statistically significant ($p = 0.0206$).

Healing of GLs/CLs and US-CPD features at 1 week

Seven of the 15 lesions classified as GLs showed the reduction of the Doppler signal associated with healing of the lesion at the final follow-up. Eight GLs presented no reduction of the US-CPD signal, and five of them healed. Fisher's exact test showed no statistical significance ($p = 0.2$). Only one of the four lesions diagnosed as CLs showed peripheral vascularity that was not present preoperatively, and this finding was not statistically related to healing ($p > 0.9999$).

Healing of GL/CL and US-CPD features at 4 weeks

Of the 15 lesions diagnosed as GLs, 12 showed a decrease/disappearance of the Doppler signal, and all of them healed, whereas the three cases that did not show modifications in the vascular features failed. There was a significant statistical association between the decrease in vascularity 4 weeks post-treatment and the healing of the lesions ($p = 0.0022$). The vascular CPD signal disappeared in the CL that exhibited the preoperative peripheral vascularity; in the CL that achieved the peripheral vascularity at 1 week, it was reduced; in a third case, the peripheral vascularity appeared for the first time; and finally, in the fourth case, there were no appreciable variations from the beginning. There was no statistical

Table 1 Assessment of the vascular flow modifications of the lesions before, during, and after endodontic treatment using ultrasound examination with color power Doppler

Case	Number of teeth	Teeth	US-CPD diagnosis	Features of vascularity	US-CPD interoperative	US-CPD postoperative	Treatment	Healing	Follow-up period (years)
1	1	28	GL	Internal	No modifications	Decrease	PRCT	Yes	7
2	1	29	GL	Internal	No modifications	Decrease	PRCT	Yes	7
3	1	10	GL	Internal	Decrease /disappearance	Decrease/disappearance	SRCT	Yes	1
4	1	27	GL	Internal	Decrease	Decrease	PRCT	Yes	7
5	1	28	GL	Internal	Decrease	Decrease	PRCT	Yes	7
6	1	19	GL	Internal	Decrease/disappearance	Decrease/disappearance	PRCT	Yes	1
7	1	3	GL	Internal	No modifications	Decrease	PRCT	Yes	1.5
8	4	23, 24,25 , 26	GL	Internal	No modifications	Decrease /disappearance	PRCT	Yes	1
9	1	26	GL	Internal	No modifications	Decrease/disappearance	PRCT	Yes	1
10	1	30	GL	Internal	Decrease /disappearance	Decrease/disappearance	PRCT	Yes	3
11	1	14	CL	Absent	No modifications	Increase	SRCT	Yes	1.5
12	1	20	CL	Absent	Increase	Decrease	SRCT	Yes	1.5
13	1	28	CL	Absent	No modifications	No modifications	PRCT	Yes	1
14	1	10	GL	Internal	No modifications	No modifications	SRCT	No	0.5
15	1	13	GL	Internal	No modifications	No modifications	SRCT	No	4
16	1	25	GL	Internal	Decrease/disappearance	Decrease/disappearance	SRCT	Yes	4
17	1	19	GL	Internal	No modifications	No modifications	SRCT	No	1
18	1	9	CL	Peripheral	No modifications	Decrease/disappearance	PRCT	Yes	0.5
19	1	19	GL	Internal	Decrease/disappearance	Decrease/disappearance	SRCT	Yes	1.5
20	3	10, 11, 12	CL	Absent	Increase peripheral	Increase peripheral	Endodontic surgery	Yes	2
21	1	14	CL	Absent	Increase peripheral	Increase peripheral	Endodontic surgery	Yes	7

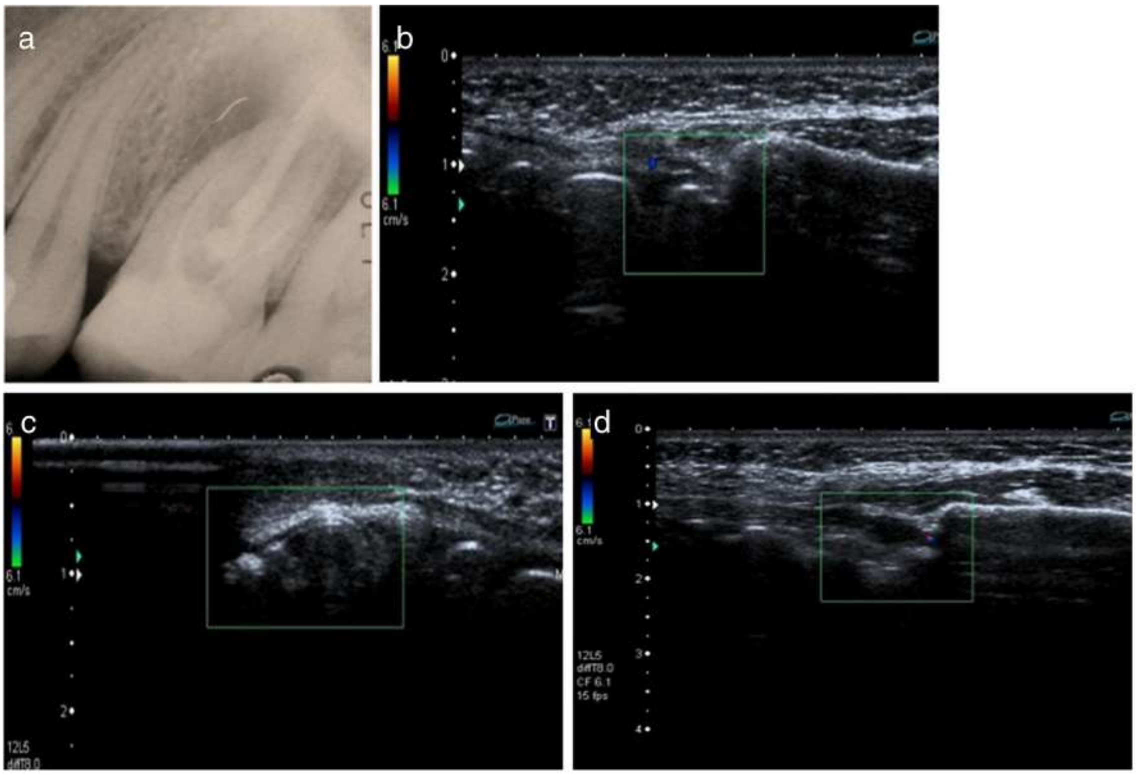

Fig. 1 Negative control. **a** Preoperative periapical radiograph showing apical periodontitis. **b** No evidence of vascular changes within the lesion from t_0, **c** at 1 week from t_0, and **d** at 4 weeks from t_0

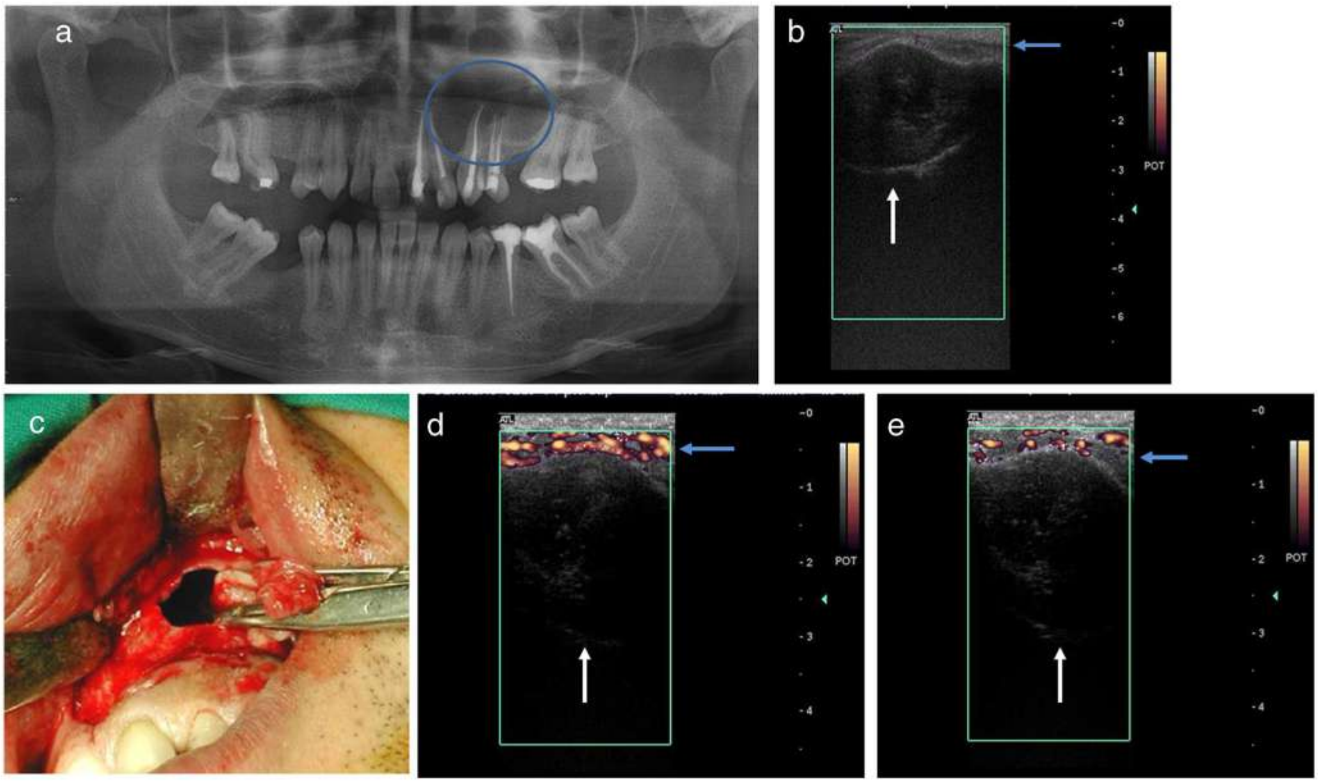

Fig. 2 Positive control. **a** Orthopantomography showing apical periodontitis involving teeth no. 10, no. 11, and no. 12 (*circled*). **b** Preoperative US-CPD showing absence of vascularity (*blue arrow*). **c** Clinical view of the endodontic surgery. **d** US-CPD at 1 week from surgery showing acute host response (*blue arrow*). **e** US-CPD at 4 weeks after the previous appointment showing a reduction in peripheral vascularity (*blue arrow*)

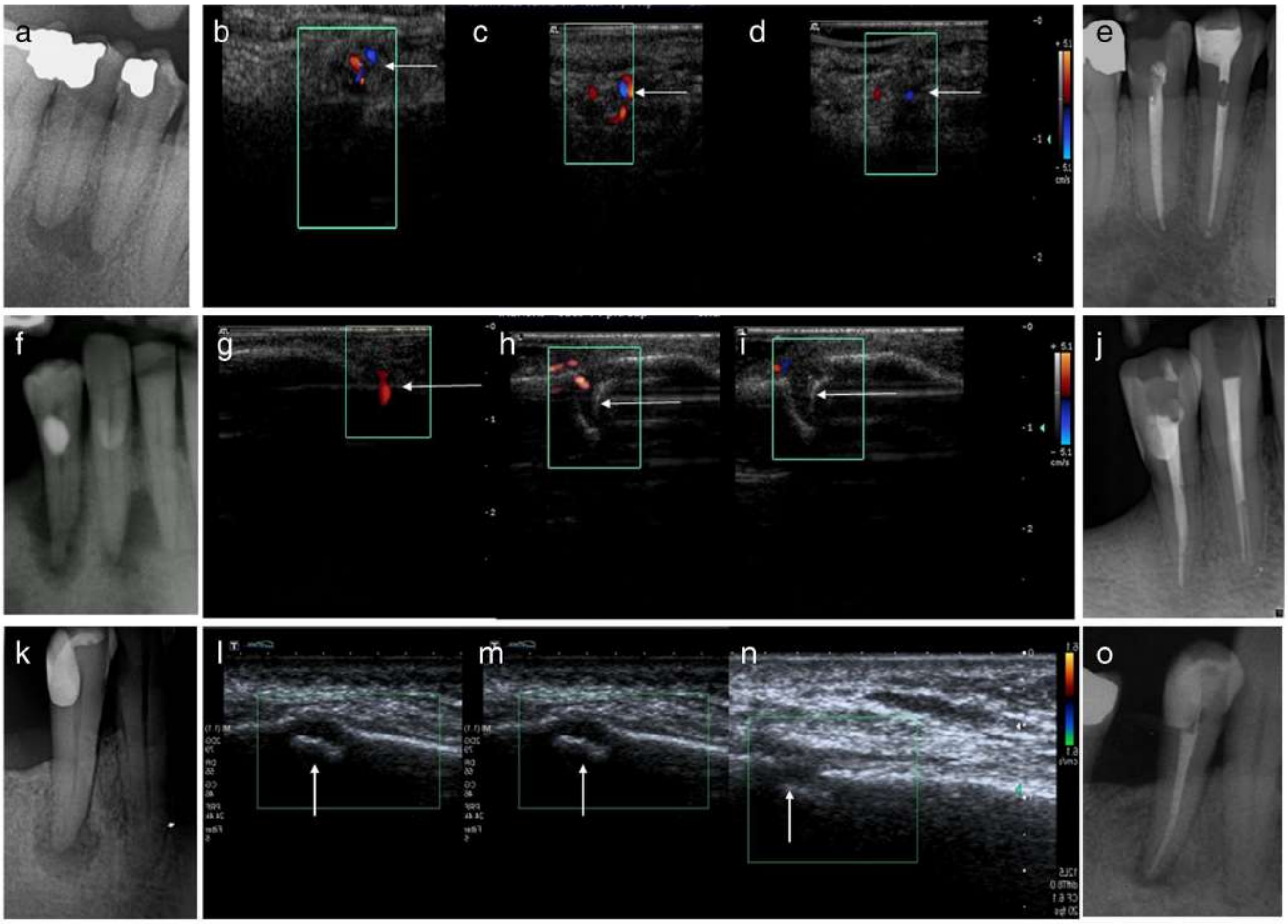

Fig. 3 Cases no. 1 and 2. **a** Periapical radiograph showing two lesions on teeth no. 28 and no. 29. **b** Preoperative US-CPD showing vascularity within the lesions (*arrow*). **c** Interoperative US-CPD showing no change in intra-lesional vascularity (*arrow*). **d** Postoperative US-CPD showing a reduction in intra-lesional vascularity (*arrow*). **e** Periapical radiograph showing healing of the lesions. Case number 4. **f** Periapical radiograph showing AP. **g** Preoperative US-CPD showing intra-lesional vascular flow (*arrow*). **h** US-CPD showing a disappearance of the intra-lesional signal (*arrow*). **i** US-CPD showing complete disappearance of intra-lesional flow at 4 weeks (*arrow*). **j** Periapical radiograph showing complete healing of the lesion. **k–o** Case no. 13. **k** Periapical radiograph showing AP. **l** Preoperative US-CPD showing absence of vascularity (*arrow*). **m** Intraoperative US-CPD showing absence of vascularity (*arrow*). **n** Postoperative US-CPD showing absence of vascularity and a decrease in diameter and increase in echogenicity (*arrow*). **o** Periapical radiograph showing a reduction in periapical index score

correlation between the vascular features of CLs and the healing trend ($p > 0.9999$).

Discussion

Little information is available on the early healing response of AP to endodontic interventions [23, 24]. Vascular response is essential in the process of bone repair [25], and the role of periodontal ligament, cementum, and alveolar bone in the healing of periapical lesions following the disinfection of the root canal system has been clearly addressed [26]. Intra-oral radiographs in conjunction with clinical findings are still crucial for the diagnosis of AP [22, 27, 28]. Although a new periapical index based on CBCT has been introduced recently [29], periapical radiography remains an accurate and reliable method in the longitudinal assessment of healing after endodontic treatment [30, 31] by detecting evidence of a change in direction from a destructive phase to a healing phase in the periapical area [32]. While traditional radiography and CBCT are used successfully in everyday practice, yet they are not able to detect modifications of the vascular supply in the periapical area [33]. However, early recognition of changes is a significant step in the appraisal of the efficacy of root canal treatment [30]. In this study, we intended to investigate the early vascular response of AP to endodontic treatment as a possible predictor of healing. US examination with CPD has already been used to monitor the long-term response (i.e., 3–6 months) to orthograde endodontic treatment. Healing lesions at 3 months postsurgery exhibited an increase in echogenicity and a decrease in volume with residual vascularity [13]. In some cases, the Doppler signal disappeared

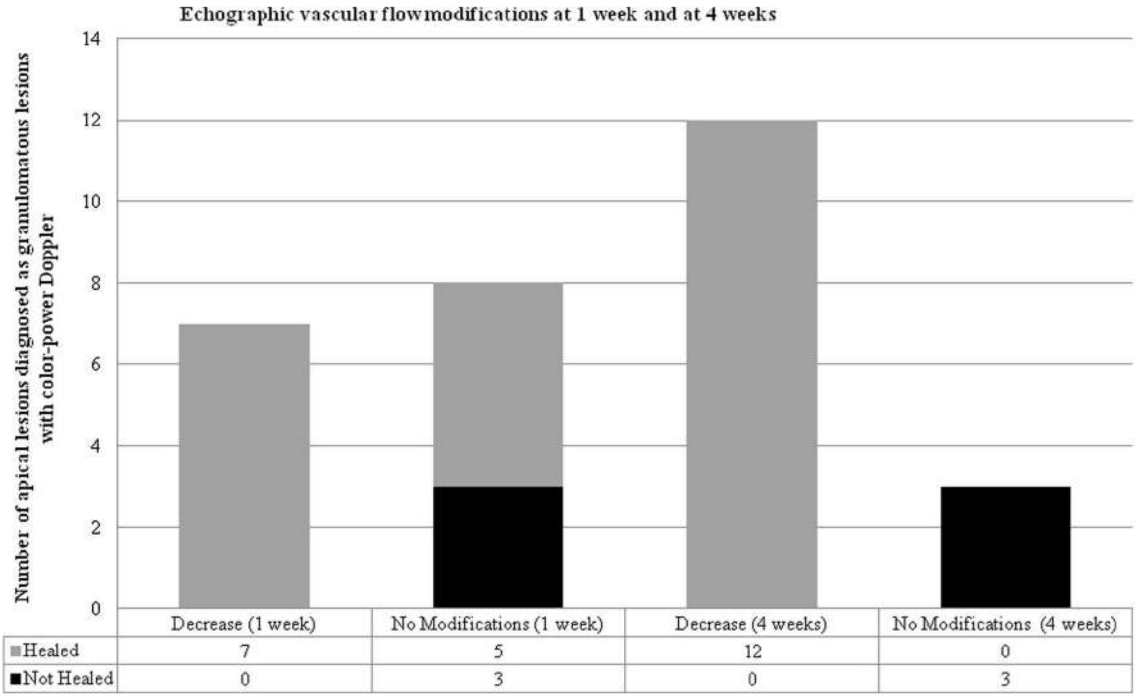

Fig. 4 Graph showing the association between the reduction in vascular flow and the healing of the apical lesions diagnosed as GLs using US-CPD

6 months after treatment as the new bone was formed [12, 13]. In this study, when the US-CPD exam was applied to a lesion of endodontic origin, without performing any treatment (negative control), no appreciable variations of the vascularity were observed at three different times.

The surgical cases were used as positive control cases, since the lesions were completely removed. These two cases exhibited initially a hypoechogenic appearance with the absence of internal vascularity at US-CPD. At 1 week following the surgical excision, both cases showed a strong peripheral Doppler signal, probably consistent with the penetration of newly formed vessels from the periphery of the surgical wound. And in the acute inflammatory phase, occurring between 0 and 2 weeks posttreatment, which starts the repair process, we also had strong peripheral Doppler signals [34]. Four weeks after surgery, the peripheral vascularity was still present with a lower CPD signal, probably due to the extinguishing of the acute inflammation and the apposition of newly formed bone [14]. We did not find a correlation between the variations in vascularity of the periapical lesions and the future healing of AP following the first access to the root canals (interoperative). The presence of irritants sustaining the inflammatory process in the periapical tissues after the first appointment may explain the lack of changes in the vascularity of AP. However, all cases that demonstrated an interoperative decrease of the vascular supply healed, suggesting that this rapid reduction in the blood flow of the lesion may be associated with the decrease of inflammation within the affected area. The modifications of the vascularity of the lesions 1 month postoperative, as detected by US-CPD, were significantly related to the healing trend of AP. Between the third and the sixth week of the healing process of the bone, a progressive diminishing of the acute inflammatory phase is expected, while the mesenchymal stromal cells are stimulated to differentiate in osteoblasts and synthesize the extracellular matrix that mineralizes [34]. Although vascularity is still present during the healing process of AP, the reduction of the inflammatory response and the progressive calcification of the damaged area lead to a decrease in the penetration of US waves and in the CPD signal until the Doppler flow disappears [12, 13]. Finally, when combining the preoperative diagnosis at the US-CPD with the vascular changes and the outcome of the endodontic treatment, it was possible to notice a different behavior between CLs and GLs (Fig. 3).

In the 15 cases echographically diagnosed as GLs [7, 35], there was a strong association between the postoperative decrease/disappearance of the CPD signal and the healing trend (Fig.4). On the other hand, the four cases diagnosed as CLs [7] did not demonstrate a correlation between the vascular changes of the lesions with their healing trend. Besides the usual lack of internal vascularity and the rare occurrence of peripheral vessels in cystic lesions [9, 36] that render such an investigation highly unpredictable, the number of lesions in this study could not facilitate the identification of any trend. US-CPD has been known to have some limitations due to the difficulty of the interpretation of the examination and to the difficulty of the US to penetrate the intact bone [37]. Actually, some of these limitations have been overcome by working in strict contact with a sonologist, using low frequencies, alternating intra-oral and extra-oral examinations, and using either a regular-sized or a small-sized probe. Furthermore, US waves have been shown to access the lesions in the maxillary bones only when they have slightly eroded either one of the cortical plates [38, 39], as happens with periapical radiographs [27, 40, 41].

Conclusion

Considering the limitations inherent in the number of cases, this study demonstrated the potential of US-CPD in imaging the early response of the patient to an endodontic treatment through the analysis of the vascular changes of the affected/treated areas. This technique, if perfected, may be used to determine the response to different treatment products and techniques. Future investigations should be conducted, and more attention should be dedicated to the potential benefits of this alternative and biologically safe imaging technique.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Funding For this type of study, funding was not required.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Moore CL, Copel JA (2011) Point-of-care ultrasonography. N Engl J Med 364:749–757. doi:10.1056/NEJMra0909487
2. Duck FA (2001) Recent epidemiology papers: european committee for medical ultrasound safety. Ultraschall Med 13:227–231. doi:10.1016/S0929-8266(01)00133-1
3. Salvesen KA (2002) Epidemiology of diagnostic ultrasound exposure during pregnancy. Ultraschall Med 15:165–171. doi:10.1016/S0929-8266(02)00038-1
4. Cotti E, Campisi G, Garau V, Puddu G (2002) A new technique for the study of periapical bone lesions: ultrasound real time imaging. Int Endod J 35:148–152. doi:10.1046/j.1365-2591.2002.00458.x
5. McDicken WN, Anderson T (2002) The difference between colour Doppler velocity imaging and power Doppler imaging. Eur J of Echocardiogr 3:240–244. doi:10.1053/euje.2002.0150
6. Martin K (2011) Basic equipment, components and image production. In: Allan PL, Baxter GM, Weston M (eds) Clinical ultrasound, 3rd edn. Churchill Livingstone, Edinburg, pp 16–30
7. Cotti E, Campisi G, Ambu R, Dettori C (2003) Ultrasound real-time imaging in the differential diagnosis of periapical lesions. Int Endod J 36:556–563. doi:10.1046/j.1365-2591.2003.00690.x
8. Gundappa M, Ng SY, Whaites EJ (2006) Comparison of ultrasound, digital and conventional radiography in differentiating periapical lesions. Dentomaxillofac Radiol 35:326–333. doi:10.1259/dmfr/60326577
9. Sumer AP, Danaci M, Sandikci EO, Sumer M, Celenk P (2009) Ultrasonography and Doppler ultrasonography in the evaluation of intraosseus lesios of the jaws. Dentomaxillofac Radiol 38:23–27. doi:10.1259/dmfr/20664232
10. Sandhu SS, Singh S, Arora S, Sandhu AK, Dhingra R (2015) Comparative evaluation of advanced and conventional diagnostic aids for endodontic management of periapical lesions, an in vivo study. J Clin Diagn Res 9:1–4. doi:10.7860/JCDR/2015/9301.5360
11. Musu D, Rossi-Fedele G, Campisi G, Cotti E (2016) Ultrasonography in the diagnosis of bone lesions of the jaws: a systematic review of the literature. Oral Med Oral Surg Oral Pathol Oral Radiol 122:e19–e29. doi:10.1016/j.oooo.2016.03.022
12. Rajendran N, Sundaresan B (2007) Efficacy of ultrasound and color power Doppler as a monitoring tool in the healing of endodontic periapical lesions. J Endod 33:181–186. doi:10.1016/j.joen.2006.07.020
13. Maity I, Kumari A, Shukla AK, Usha HL, Naveen DN (2011) Monitoring of healing by ultrasound with color power doppler after root canal treatment of maxillary anterior teeth with periapical lesions. J Conserv Dent 14:252–257. doi:10.4103/0972-0707.85804
14. Tikku AP, Kumar S, Loomba K, Chandra A, Verma P, Aggarwal R (2010) Use of ultrasound, color Doppler imaging and radiography to monitor periapical healing after endodontic surgery. J Oral Sci 52:411–416. doi:10.2334/josnusd.52.411
15. Dessi C, Demartis P, Campisi G, Cotti E (2009) Use of ultrasound real-time examination and colour –doppler to evaluate initial response to root canal treatment (Abstract). Int Endod J 42:1139
16. Jafarzadeh H, Abbott PV (2010) Review of pulp sensibility tests. Part I: general information and thermal tests. Int Endod J 43:738–762. doi:10.1111/j.1365-2591.2010.01754.x
17. Bhaskar SN (1966) Periapical lesions-types, incidence, and clinical features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 21:657–671
18. Goldman M, Pearson A, Darzenta N (1974) Reliability of radiographic interpretations. Oral Surg Oral Med Oral Pathol 38:287–293
19. Ramfjord SP (1967) The Periodontal Disease Index (PDI). J Periodontol 38:602–610. doi:10.1902/jop.1967.38.6.602
20. AAE & AAOM (2015) AAE and AAOM joint position statement American Association of Endodontists/American Academy of Oral and Maxillofacial Radiology (online). Use of cone beam computed tomography in endodontics. https://www.aae.org/uploadedfiles/clinical_resources/guidelines_and_position_statements/cbctstatement_2015update.pdf. Accessed August 17 2016
21. Kim S, Kratchman S (2006) Modern endodontic surgery concepts and practice: a review. J Endod 32:601–623. doi:10.1016/j.joen.2005.12.010
22. Ng YL, Mann V, Gulabivala K (2011) A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. Int Endod J 44:583–609. doi:10.1111/j.1365-2591.2011.01872.x
23. Nair PNR (2006) On the cause of persistent apical periodontitis: a review. Int Endod J 39:246–281. doi:10.1111/j.1365-2591.2006.01099.x
24. Lin L, Chen MYH, Ricucci D, Rosenberg PA (2010) Guided tissue regeneration in periapical surgery. J Endod 36:618–625. doi:10.1016/j.joen.2009.12.012
25. Saran U, Gemini Piperni S, Chatterjee S (2014) Role of angiogenesis in bone repair. Arch Biochem Biophys 561:109–107. doi:10.1016/j.abb.2014.07.006
26. Marton IJ, Kiss C (2014) Overlapping protective and destructive regulatory pathaways in apical periodontitis. J Endod 40:155–163. doi:10.1016/j.joen.2013.10.036

27. Bender IB, Seltzer S (1961) Roentgenographic and direct observation of experimental lesions in bone. J Am Dent Assoc 87:708–716
28. Kruse C, Spin-Neto R, Wenzel A, Kirkevang LL (2015) Cone beam computed tomography and periapical lesions: a systematic review analysing studies on diagnostic efficacy by a hierarchical model. Int Endod J 48:815–828. doi:10.1111/iej.12388
29. Estrela C, Bueno MR, Azevedo BC, Azevedo JR, Pècora JD (2008) A new periapical index based on cone beam computed tomography. J Endod 34:1325–1331. doi:10.1016/j.joen.2008.08.013
30. Delano EO, Ludlow J, Ørstavick D, Tyndall D, Trope M (2001) Comparison between PAI and quantitative digital radiographic assessment of apical healing after endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 92:108–115. doi:10.1067/moe.2001.115466
31. Kirkevang LL, Ørstavik D, Wenzel A, Væth M (2015) Prognostic value of the full-scale periapical index. Int Endod J 48:1051–1058. doi:10.1111/iej.12402
32. Ørstavik D, Kerekes K, Eriksen HM (1986) The periapical index: a scoring system for radiographic assessment of apical periodontitis. Endod Dent Traumatol 2:20–24. doi:10.1111/j.1600-9657.1986.tb00119.x
33. Guo J, Simon JH, Sedghizadeh P, Soliman ON, Chapman T, Enciso R (2013) Evaluation of the reliability and accuracy of using cone-beam computed tomography for diagnosing periapical cysts from granulomas. J Endod 39:1485–1490. doi:10.1016/j.joen.2013.08.019
34. Folestad A, Alund M, Asteberg S et al (2015) Offloading treatment is linked to activation of proinflammatory cytokines and start of bone repair in Charcot arthropathy patients. J Foot Ankle Res 72: 1–12. doi:10.1186/s13047-015-0129-y2015
35. Raghav N, Reddy SS, Giridhar AG et al (2010) Comparison of the efficacy of conventional radiography, digital radiography and ultrasound in diagnosis of periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 110:379–385. doi:10.1016/j.tripleo.2010.04.039
36. Cotti E, Simbola V, Dettori C, Campisi G (2006) Echographic evaluation of bone lesions of endodontic origin: report of two cases in the same patient. J Endod 32:901–905. doi:10.1016/j.joen.2006.01.013
37. Patel S, Dawood A, Whaites E, Pitt Ford T (2009) New dimensions in endodontic imaging: part 1. Conventional and alternative radiographic systems. Int Endod J 42:447–462. doi:10.1111/j.1365-2591.2008.01530.x
38. Hayashi T (2012) Application of ultrasonography in dentistry. Jpn Dent Sci Rev 48:5–13. doi:10.1016/j.jdsr.2011.05.001
39. Sakuma K, Ikarugi Y, Tanaka R, Hayashi T (2007) Ultrasonography of periapical lesion: comparison with CT. Dental Radiol 47:53–64
40. van der Stelt PF (1985) Experimentally produced bone lesions. Oral Surg Oral Med Oral Pathol 59:306–312
41. Marmary Y, Koter T, Heling I (1999) The effect of periapical rarefying osteitis on cortical and cancellous bone. A study comparing conventional radiographs with computed tomography. Dentomaxillofac Radiol 28:267–271. doi:10.1038/sj/dmfr/4600453

Chapter 5

Ultrasound examination to visualize and trace sinus tracts of endodontic origin

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CLINICAL RESEARCH

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Ultrasound Examination to Visualize and Trace Sinus Tracts of Endodontic Origin

SIGNIFICANCE

Ultrasound real-time examination is a promising tool to detect and trace noninvasively sinus tracts of endodontic origin. It produces a direct image rather than one obtained with a gutta-percha cone inserted into the tract. The vascular reaction within and around the tract can be also observed by the 3D rendering and the color and power Doppler implementation.

ABSTRACT

Introduction: The detection of a tooth with a sinus tract (ST) of endodontic origin and its pathway are conventionally assessed with a periapical radiograph and a gutta-percha cone introduced into its stoma. The aim of this study was to evaluate the possibility to detect STs and trace their route using ultrasound real-time examination. **Methods:** Two calibrated examiners performed echography on 10 patients who had a lesion of apical periodontitis (AP) and ST and 10 patients in the control group with AP without an ST recruited in 2 endodontic practices. They also traced the pattern of the STs with a computer program. The images were then submitted to 2 calibrated and blinded observers who were asked to describe the presence of AP and ST and to trace it with the same program. The data obtained were compared with the clinical and radiographic diagnosis of ST. For sensitivity, specificity, accuracy, and positive and negative predictive values, the receiver operating characteristic curve and Fisher exact test were used ($P < .05$). **Results:** Interobserver agreement was high as was the diagnostic accuracy of the ultrasound examination of STs (mean value = 97.5%), and the Fisher exact test showed statistical significance ($P < .05$). High sensitivity and a negative predictive value and 100% specificity and a positive predictive value were also obtained. The application of the 3-dimensional mode further enabled the reconstructions of the more complex paths, and the implementation with color power Doppler disclosed the vascularity surrounding the STs. **Conclusions:** The ultrasound examination is a technique feasible to describe and trace the STs of endodontic origin. *(J Endod 2019;45:1184–1191.)*

KEY WORDS

Apical periodontitis; oral radiology; sinus tract; ultrasound examination

A sinus tract (ST) of endodontic origin is a pathway from an enclosed area of infection (eg, a root canal) to an epithelial surface through an opening (or stoma), which can be intraoral or extraoral[1]. The presence of apical periodontitis (AP) associated with an ST is classified as a chronic apical abscess[2,3], and the prevalence of this condition ranges from 7.4%–30.75%[4–7]. The degree of epithelialization of the tract is still debated because of its tortuous nature[8]. Patients presenting with an ST are usually asymptomatic, with the draining fistulous opening as the only chief complaint[9]. The ST is expected to heal when the source of the endodontic infection is eliminated after the root canal treatment or the extraction of the offending tooth[10,11]. The identification of the tooth responsible for the ST can be complicated by its opening at a distant site or by the presence of multiple stomas[6], and when STs open in the skin of the face and neck, they can be easily misdiagnosed as dermatologic diseases[9,12,13]. The detection of the infected tooth and the pathway of the ST from the root canal to the stoma are conventionally assessed by taking a periapical radiograph after introducing, in its orifice, a gutta-percha cone[14,15] or a fine stainless steel orthodontic wire[6,16]. Ultrasound (US) real-time examination is a noninvasive imaging technique extensively used in medicine based on the propagation and reflection of acoustic waves across the different tissues of the body[17,18]. In recent times, US has found a wide application in the examination of the head and neck district[19] and in the assessment, differential diagnosis, and follow-up of AP after endodontic treatment[20–23]. The aim of this study was to evaluate the possibility to detect STs of endodontic origin and trace their route using US real-time examination, also called echography.

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MATERIALS AND METHODS

The present protocol was conducted in accordance with the institutional ethics committee and the Declaration of Helsinki 1975 (as revised in 2000, PG/2017/9991). The subjects participating in this study were selected among the patients referred to 2 local endodontic practices. After approval for participating in the present study, the patients were asked to sign an informed consent form and were recruited in the research protocol according to the inclusion/exclusion criteria.

Inclusion Criteria

Healthy patients not taking any medication with at least 1 tooth with AP[24] with a periapical index score (PAI)[25] of 3 or more with or without an ST were included in this study.

Exclusion Criteria

Pregnant women, patients with systemic diseases taking medications, patients with a fracture or periodontitis diagnosed on the tooth with the ST, and patients not willing to participate to the study were excluded.

Diagnosis of AP

After assessment of the medical and dental history, the patients received a thorough dental examination. Endodontic clinical evaluation was performed along with the following tests: palpation, percussion, occlusal evaluation, tooth mobility, periodontal probing, bite test, and pulp sensitivity tests for each tooth. Two or more intraoral periapical radiographs were also performed[26]. The diagnosis of ST was done through direct observation of a stoma (draining or not draining), and a periapical radiograph was performed positioning a gutta-percha cone within the tract[12,24].

The assessment of PAI was performed by the postgraduate endodontic students following the tables of Ørstavik et al[25] at the Department of Conservative Dentistry and Endodontics, University of Cagliari, Cagliari, Italy. The decision had to be confirmed by the head of the department. For the aim of the present study, the PAI score was used as a diagnostic decisional tree where the patients were dichotomized in PAI ≥ 3 and PAI <3.

Subject Material

Twenty white patients (20 teeth) affected by AP (8 men and 12 women, mean age = 47.2) were divided into the following 2 groups:

1. The cases (APST): 10 teeth with 1 lesion of AP with an ST
2. The controls (CT): 10 teeth with 1 lesion of AP without an ST

The cases and controls were selected by 2 examiners (an expert sonologist and a trained expert endodontist) and examined by 2 blinded observers.

US Examination

The 2 examiners performed calibration with the images of STs collected from a previous pilot study (A. Goddi, 2017), and these samples were not included in the main experiment. The level of agreement among the observers was calculated through kappa statistics. The 2 blinded observers performed calibration exercises with the images of cases of AP, with and without an ST, from the previous pilot study until they obtained a good agreement (Cohen kappa of 0.3, 0.67, and 1.00, at the first, second, and third examination, respectively). Two weeks later, the same images were submitted for further examination to calculate the intraexaminer agreement. All US examinations were performed using either an Elegra (Siemens, Erlangen, Germany) or an Aplio XG (Toshiba Medical Systems, Crawley, UK) apparatus with regular and small-size, linear, high-definition, multifrequency US probes. To have the availability of the 2 units during the main experiment and to not produce confounding data, the 2 linear multifrequency probes of 7–9 MHz and 8–12 MHz, were respectively coupled with the Elegra Siemens and the Toshiba Aplio XG to generate equivalent output scans during the calibration. After the application of an echographic gel, the probe was placed and moved both extraorally on the skin of the upper and lower jaw and intraorally in the area of the alveolar mucosa, corresponding to the roots of the involved teeth. The position of the probe was changed several times to obtain an adequate and representative number of scans. In addition, the color power Doppler (CPD) was applied to all APST cases to assess the presence, amount, and features of the vascular supply within or outside the tract. Once several scans were acquired from each case, the data were converted into 3-dimensional (3D) images. The representative images were saved as TIFF files, organized on a PowerPoint presentation (Microsoft Office 2013; Microsoft Corporation, Redmond, WA) on the screen of a Dell Inspiron 15 laptop computer (Dell Inc, Round Rock, TX), and submitted to the blinded observers separately and without the radiograph where the ST was traced. The blinded observers were then asked to diagnose independently the presence or absence of an ST associated with AP and to fill a dedicated chart. In addition, they were asked to draw the route of the ST on the images using the same computer program. The diagnosis of ST in this study was considered valid only if the fistulous pathway was traced by both observers and if it was possible to superimpose the 2 images. The observers repeated the same examination 2 weeks later.

Data Analysis

The data from the ultrasonic examinations were then compared with the clinical and radiographic diagnosis of ST to assess sensitivity, specificity, accuracy, positive predictive value, and negative predictive value and to establish the reliability of the technique. The accuracy of the procedure was also evaluated with the receiver operating characteristic curve (area under the curve). Given the small number of samples observed in this study, the Fisher exact test was used coherently with the sample size to calculate the significance of agreement on the echographic analysis according to the clinical and radiographic diagnoses ($P < .05$). The Fisher exact test requires 2×2 contingency tables, a number of observations <30, and cells with expected frequencies ≤ 5.

RESULTS

All 20 patients did not experience any discomfort during the US examination. The examiners detected AP in all US examinations (APST and CT groups) and traced all 10 STs in the APST group (Table 1). The US images of AP in the CT group were anechoic to hypoechoic areas, developing deeply under a well-defined hyperechoic band corresponding to the buccal cortical bone plate. In the presence of AP, the buccal plate was either perforated or thinned (depending on the scan), creating an acoustic window for the US waves to access the lesions. A posterior acoustic reinforcement (white rim) where the UT could not penetrate any further was the common additional feature at the deepest part of the lesion[27] (Fig. 1*A* and *B*). In the echographic images of all APST cases, there was a detectable interruption of the cortical bone plate (Fig. 2*A–F*) necessary for the discharge of exudation from the lesion to the soft tissues. In the B-mode US images, the STs appeared as dishomogeneous, hypoechoic pathways lined by echogenic and reinforced walls, connecting the lesion to the intra- or extraoral tissues

TABLE 1 - Data Collection of the Apical Periodontitis (AP) Lesions Investigated by Ultrasound (US) Real-time Examination

Patient no.	Sex	Age	Teeth	US diagnosis	Diagnostic group	Doppler signal	3D modality
1	F	50	3.4	AP with ST	Case	Absent	Not performed
2	F	25	1.2, 1.1	AP with ST	Case	Present	Performed
3	F	56	3.6	AP with ST	Case	Absent	Not performed
4	M	57	3.6	AP with ST	Case	Present	Performed
5	F	35	1.6	AP with ST	Case	Present	Performed
6	M	52	1.2	AP with ST	Case	Present	Performed
7	F	54	3.7	AP with ST	Case	Present	Performed
8	M	57	1.6	AP with ST	Case	Absent	Performed
9	F	50	3.4, 3.5	AP with ST	Case	Present	Performed
10	F	42	1.6	AP with ST	Case	Absent	Not performed
11	F	24	3.6	AP	Control	Absent	Not performed
12	M	53	1.1, 1.2	AP	Control	Absent	Not performed
13	F	73	1.2	AP	Control	Present	Performed
14	M	68	4.3	AP	Control	Present	Performed
15	M	37	3.6	AP	Control	Absent	Performed
16	M	24	4.7	AP	Control	Present	Not performed
17	F	70	23	AP	Control	Absent	Not performed
18	M	50	2.1	AP	Control	Absent	Not performed
19	F	25	2.6	AP	Control	Absent	Not performed
20	F	42	2.5	AP	Control	Absent	Not performed

3D, 3-dimensional; F, female; M, male; ST, sinus tract.

where the stoma opened (9 cases and 1 case, respectively). When these tracts reached the alveolar mucosa, they were framed by an echogenic area at the interface between the buccal oral tissues and the cortical plate (Fig. 2). In the scans of the extraoral ST, which generated from tooth #19 and opened at the skin surface of the right mandible, the hypoechoic band crossed the muscular layer of the buccinator to reach the surface (Fig. 3*A–F*). The observers diagnosed the absence of an ST in all the images of the CT group and highlighted the presence and route of an ST in 9 out of 10 images in the APST group. In 1 case, there was a mismatch between the observers in the tracing of the ST path, and the diagnosis of ST could not be validated. After a consensus with the nonblinded examiners was reached, 1 of the pathways was considered correct. In 6 of the APST cases, the whole ST could be entirely framed within a single scan. However, in 4 cases (patients #4, 6, 7, and 8), the pathway of the ST was particularly tortuous and required more scans to be traced. In these cases, a 3D image of the entire tract was obtained with the 3D elaboration mode (3D rendering multislice view) (Fig. 4*A–F*). The results from the CPD showed no vascular signal within the lumen of all the tracts; however, in 5 patients[2,4–7], small vessels were identified along the echogenic walls of the ST, revealing a possible inflammatory reaction (Fig. 4). At the first examination, the level of interobserver agreement was very high (Cohen $\kappa = 0.9$). The 2 observers confirmed the results 2 weeks later (Cohen $\kappa = 0.9$). In addition, the

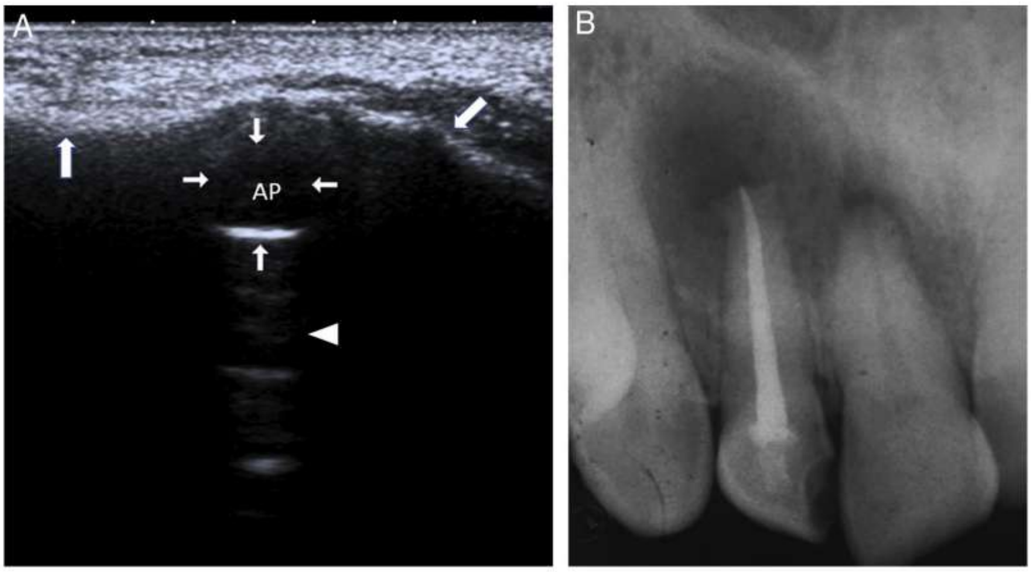

FIGURE 1 – (*A*) An echographic image of AP showing an intact cortical bone plate (*arrows*) and the posterior acoustic reinforcement (*arrowhead*). (*B*) A periapical radiograph depicting the same lesion (tooth #7).

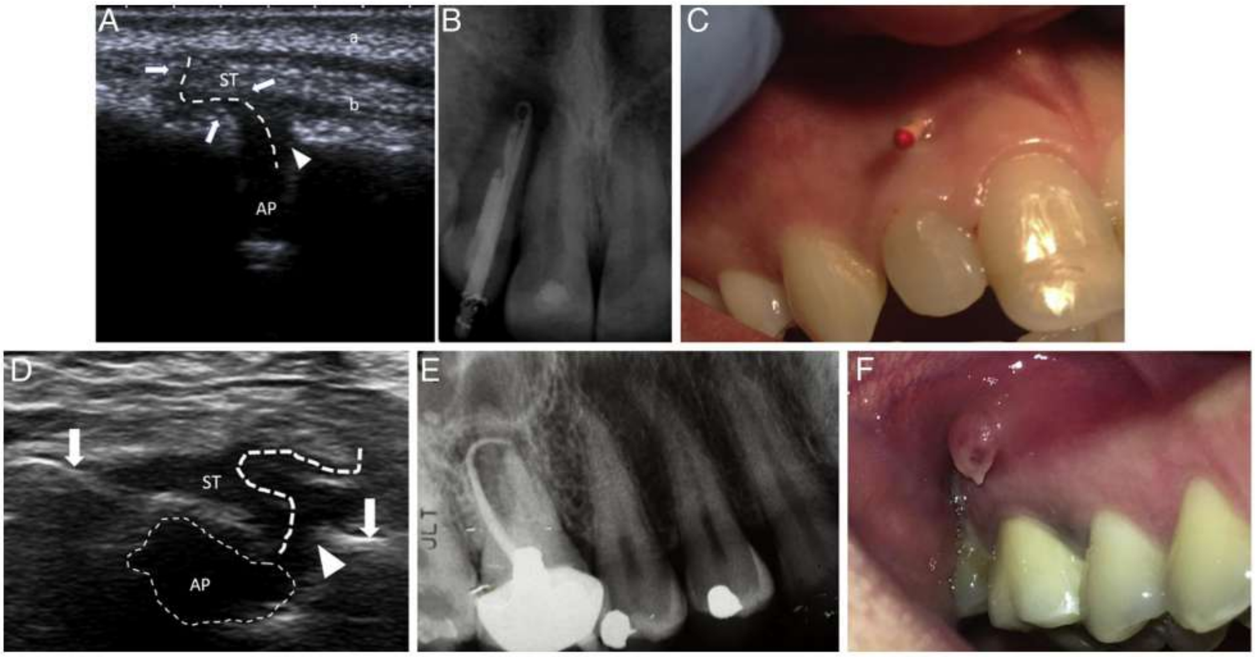

FIGURE 2 – (*A*) An ecographic representation of an ST interrupting the cortical bone plate (*arrowhead*) depicted as a dishomogeneous, hypoechoic pathway (*interrupted line*) lined by echogenic and reinforced walls (*arrows*). (*B*) The same ST traced with a periapical radiograph (tooth #7). (*C*) The corresponding clinical image. (*D*) Ecographic tracing of another ST interrupting the cortical bone plate (*arrows*) through a breach (*arrowhead*). (*E*) The same ST traced with a periapical radiograph (tooth #3). (*F*) A clinical image of the corresponding hypertrophic stoma.

intraobserver agreement calculated 2 weeks later showed a kappa value of 1.00. This technique showed high sensitivity and a negative predictive value and 100% specificity and a positive predictive value (Table 2). According to the receiver operating characteristic curve analysis (Fig. 5), the diagnostic accuracy of the US examination to detect STs had a mean value of 97.5% in the blinded observers. In addition, the Fisher exact test showed a statistical significance for both observers ($P < .05$, Table 2).

DISCUSSION

Achieving new and more complete information on STs is of primary interest in clinical endodontics because the presence of a preoperative ST has been reported to reduce the odds of success of periapical

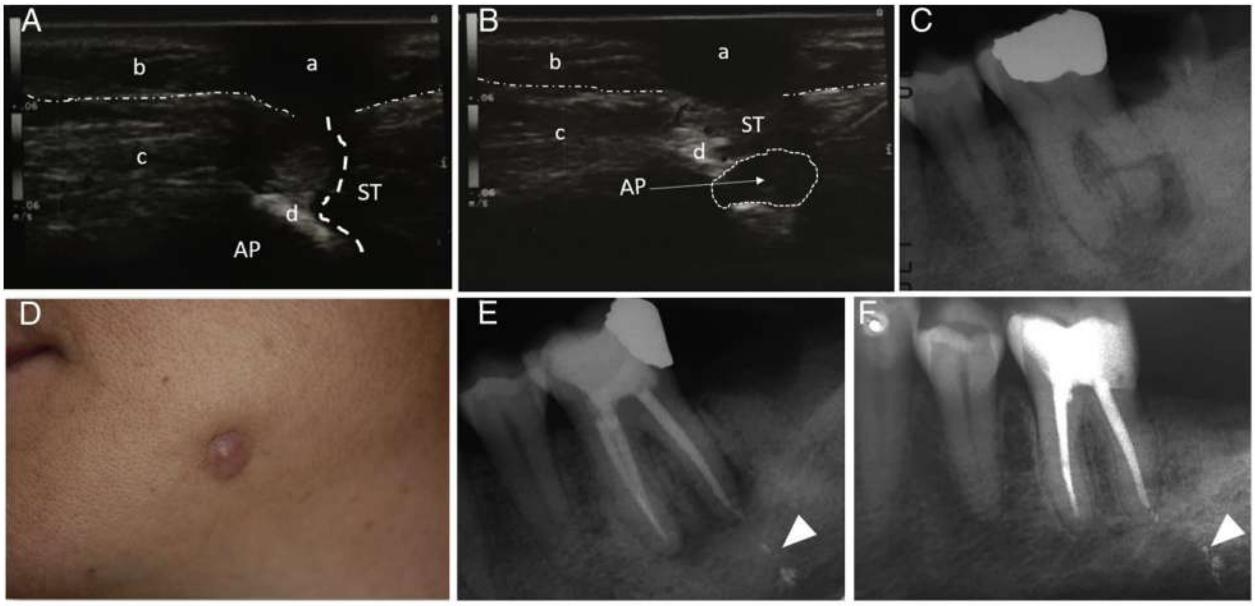

FIGURE 3 – (*A*) An ecographic image of an extraoral ST (*interrupted line*) representing the (*a*) opening at the (*b*) skin surface (*fragmented contour*), the (*c*) muscular layer, and the (*d*) mandibular bone. (*B*) Another perspective of the same lesion showing the whole AP (*interrupted contour*). (*C*) A periapical radiograph of the same lesion on tooth #18. (*D*) Clinical detail of the cutaneous stoma. (*E*) A postoperative radiographic image showing the extruded root canal sealer following the ST (*arrowhead*). (*F*) A 3-month postoperative radiograph showing the progressive movement of the sealer (*arrowhead*) and healing of the lesion.

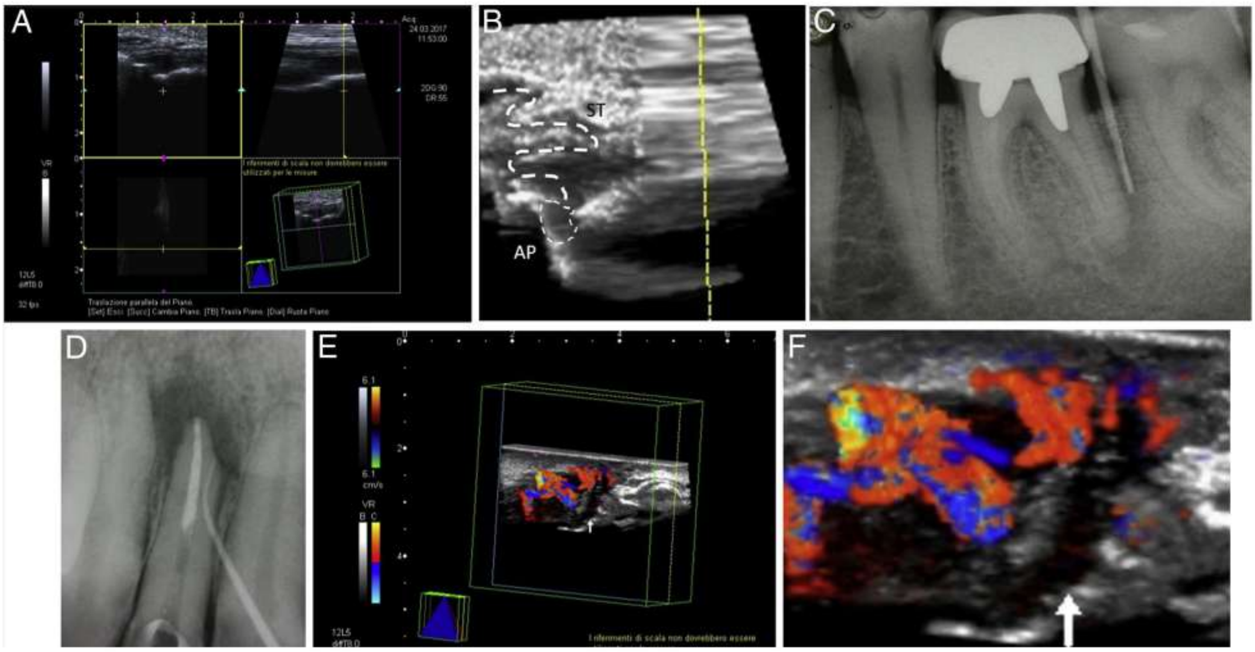

FIGURE 4 – (*A*) An APST case visualized in the 3D elaboration mode. (*B*) Tridimensional reconstruction of AP (*interrupted contour*) and the ST (*interrupted line*). (*C*) A periapical radiograph of the same case and tracing of the tract (tooth #18). (*D*) An APST case traced with a gutta-percha cone and a periapical radiograph (tooth #7). (*E*) Evaluation of the vascularity in the same case using CPD. (*F*) CPD detail showing the ST (*arrow*) and identifying the presence of small vessels at the walls of the ST lumen.

healing by 48%[28] and to increase the hazard of tooth loss by 120%[29]. The difficulty in the healing of STs has been explained in the past with the presence of an epithelial lining throughout the tract[8,30], which would hamper the closure of the pathologic pathway despite the adequate treatment of the root canal infection. Epithelialization as the cause of failure has been dispelled by the evidence that the persistence of infection was a more important reason for the lack of resolution of the ST[11,31]. Concentrations above the average of specific pathogens in intraradicular infection have been considered responsible for the poorer prognosis of teeth with chronic apical abscesses; yet, no significant differences were found between cases with and without STs in terms of total bacterial counts[32]. Finally, the persistence of STs has been attributed to the presence of extraradicular infections such as actinomycosis[33], but there is still no definite evidence that an extraradicular infection alone can sustain refractory periapical inflammation[34,35]. Indeed, in a recent histobacteriologic study on 24 biopsy specimens from roots of teeth associated with AP and an ST[36], the authors highlighted the complexity of the infection that characterized those cases. They reported that 83% of the samples had a compound infectious pattern that involved the canal system, the periapical lesion, and the extraradicular fraction of the root, the latter always derived from the intraradicular biofilm present in all canals. To our knowledge, this is the first experimental study designed to assess the potential of US to scan STs, track their pathways, and evaluate their vascularity. Only 1 article in the field of dermatology has reported the application of US in 3 cases of cutaneous STs of endodontic origin at first misdiagnosed and overtreated with invasive and unnecessary interventions. In the mentioned study, a linear high-frequency US probe was used to investigate the lesions in the skin; the tracts were described as hypoechoic bands connecting the skin to the alveolar bone with a distinguished CPD signal at the periphery of 2 lesions, as observed in some of our cases, yet they did not highlight the pathway of the STs[37]. The results of this study showed that US examination was feasible to visualize and track the path of an ST of endodontic origin. The common procedure used to trace a chronic apical abscess contemplated the insertion of a gutta-percha cone through the opening of the ST followed by a periapical radiograph of the area to achieve an indirect image of the tract (Fig. 2*B* and *E*)[14,16]. With US imaging, the route of drainage of the fluid can be completely imaged from the periapical lesion to the stoma of the ST (Figs. 2 and 3). The reconstruction of the images with the 3D mode can also disclose 3-dimensionally the pathway of the tract and illustrate its irregularities and curves. The implementation of the examination with CPD further allows the assessment of the vascular pattern and, consequently, the inflammatory response of the tissues surrounding the ST. Moreover, the technique has the serious advantage of not using ionizing radiations[38]. The choice of a linear multifrequency probe in this experiment

TABLE 2 - Data Analysis of the Results Obtained by the Blinded Observers during the Different Examinations

Observer	Examination	Sensibility	Specificity	PPV	NPV	ROC	Significance ($P < .05$)
Observer 1	1	100	100	100	100	100	$P = .0$
Observer 2	1	90	100	100	91	95	$P = .001$
Observer 1	2	100	100	100	100	100	$P = .0$
Observer 2	2	90	100	100	91	95	$P = .001$

NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic.

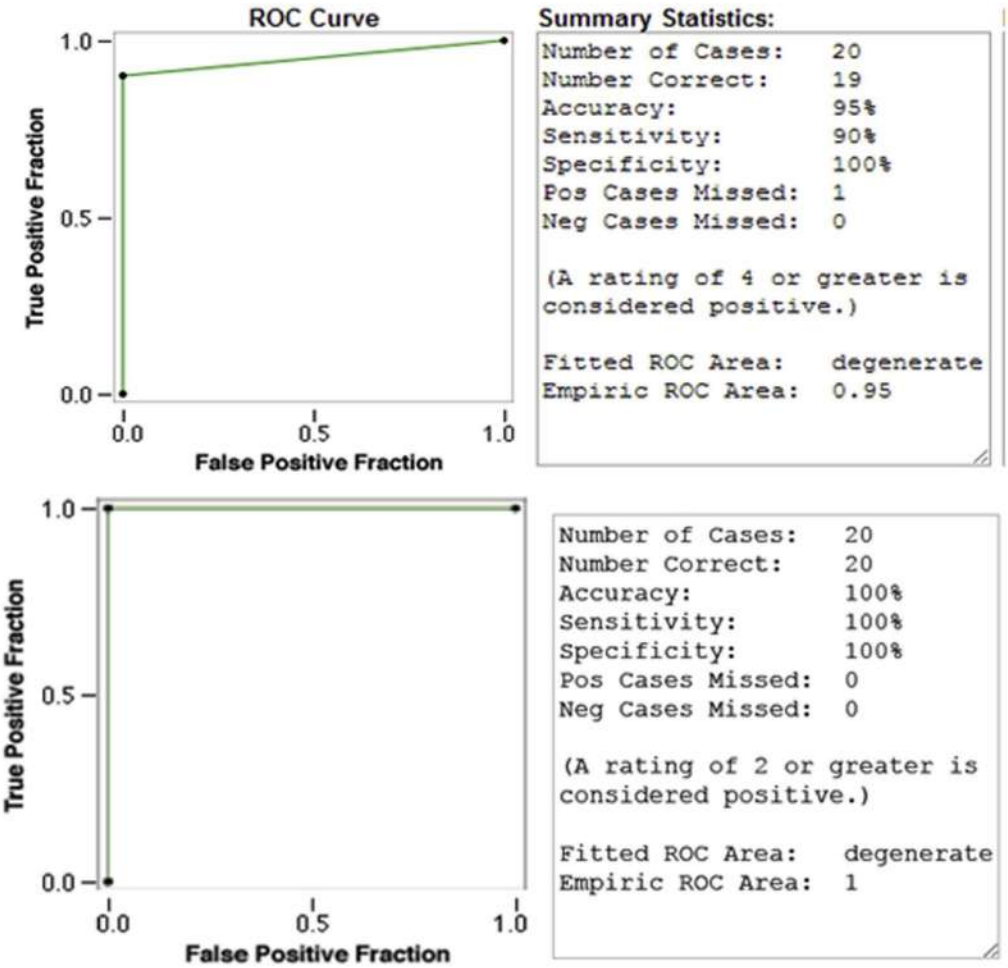

FIGURE 5 – Receiver operating characteristic curve (area under the curve) analysis showing the diagnostic accuracy of the technique for the blinded observers.

is justified because lower frequencies can penetrate deeper within the bone defect, whereas higher frequencies permit visualization of the soft tissues and the STs with a better definition[39]. Although the examination should be performed in real time to achieve the most from its diagnostic power, the analysis of the images performed from the blinded examiners after their acquisition showed high values of sensibility, specificity, and accuracy (Table 2). The time necessary to obtain the US image of the ST on a selected area is longer than making a radiograph; on the other hand, once the image is obtained, using the CPD or the 3D extension is relatively fast.

The small number of cases constitutes 1 of the main limitations of the statistical significance of the present study, and in order to determine the power of the associations of the observations, we have performed a statistical test of significance. One of the reported limitations of US examination is that it requires a learning curve and that the interpretation of the images necessitates training and a certain level of experience[23,27]. Nevertheless, good training may enable most clinicians to perform and analyze the images because the anatomic layers that need to be crossed by the US waves to reach the lesions and the STs are the skin, the alveolar mucosa, and the cortical bone plate.

CONCLUSION

US real-time examination can be successfully used to detect the STs of endodontic origin and to trace their route of drainage from the periapical lesion to the opening within the oral mucosa or the skin. Future studies are needed to standardize the technique. To date, the importance of this achievement may be limited to the descriptive diagnostic field.

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The authors deny any conflicts of interest related to this study.

REFERENCES

1. American Association of Endodontists. Glossary of Endodontic Terms. 9th ed. American Associations of Endodontists (AAE); 2016. Available at: http://www.nxtbook.com/nxtbooks/aae/endodonticglossary2016/index.php. Accessed October 9, 2018.
2. Abbott PV. Classification, diagnosis and clinical manifestations of apical periodontitis. Endod Topics 2004;8:36–54.

3. Torabinejad M, Shabahang S. Pulp and periapical pathosis. In: Torabinejad M, Walton RE, Fouad AF, editors. Endodontics. Principles and Practice. 5th ed. St Louis, MO: Saunders/Elsevier; 2015. p. 48–67.

4. Gupta R, Hasselgren G. Prevalence of odontogenic sinus tracts in patients referred for endodontic therapy. J Endod 2003;12:798–800.

5. Miri SS, Atashbar O, Atashbar F. Prevalence of sinus tract in the patients visiting department of endodontics, Kermanshah school of dentistry. Glob J Health Sci 2015;6:271–5.

6. Slutzky-Goldberg I, Tsesis I, Slutzky H, Heling I. Odontogenic sinus tracts: a cohort study. Quintessence Int 2009;40:13–8.

7. Soğur E, Sen HB, Baksı GB, Mert A. Prevalence of odontogenic sinus tracts in patients referred for endodontic therapy. Cumhuriyet Dent J 2013;16:282–8.

8. Vire DE, Stalker WH, Kessler HP. Epithelium-lined oral sinus tract. Oral Surg 1982;53:209–11.

9. Mittal N, Gupta P. Management of extra oral sinus cases: a clinical dilemma. J Endod 2004;30:541–7.

10. McWalter GM, Alexander JB, del Rio CE, Knott JW. Cutaneous sinus tracts of dental etiology. Oral Surg 1988;66:608–14.

11. Spear KL, Sheridan PJ, Perry HO. Sinus tracts to the chin and jaw of dental origin. J Am Acad Dermatol 1983;8:486–92.

12. Gupta M, Das D, Kapur R, Sibal N. A clinical predicament—diagnosis and differential diagnosis of cutaneous facial sinus tracts of dental origin: a series of case reports. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:e132–6.

13. Heling I, Rotstein I. A persistent oronasal sinus tract of endodontic origin. J Endod 1989;15:132–4.

14. Baumgartner JC, Picket AB, Muller JT. Microscopic examination of oral sinus tracts and their associated periapical lesions. J Endod 1984;10:146–52.

15. Johnson BR, Remeikis NA, Van Cura JE. Diagnosis and treatment of cutaneous facial sinus tracts of dental origin. J Am Dent Assoc 1999;130:832–6.

16. Bender IB, Seltzer S. The oral fistula: its diagnosis and treatment. Oral Surg Oral Med Oral Pathol 1961;64:139–48.

17. Moore CL, Copel JA. Point-of-care ultrasonography. N Engl J Med 2011;364:749–57.

18. Miller DL. Safety assurance in obstetrical ultrasound. Semin Ultrasound CT MR 2008;29:156–64.

19. Mohan KR, Koteswara RN, Leela KG, et al. Role of ultrasonography in oral and maxillofacial surgery: a review of literature. J Maxillofac Oral Surg 2015;14:162–70.

20. Cotti E, Simbola V, Dettori C, Campisi G. Echographic evaluation of bone lesions of endodontic origin: report of two cases in the same patient. J Endod 2006;32:901–5.

21. Rajendran N, Sundaresan B. Efficacy of ultrasound and color power Doppler as a monitoring tool in the healing of endodontic periapical lesions. J Endod 2007;33:181–6.

22. Cotti E, Esposito SA, Musu D, et al. Ultrasound examination with color power Doppler to assess the early response of apical periodontitis to the endodontic treatment. Clin Oral Investig 2018;22:131–40.

23. Curvers F, Meschi N, Vanhoenacker A, et al. Ultrasound assessment of bone healing after root-end surgery: echoes back to patient's safety. J Endod 2017;44:32–7.

24. Hargraves K, Berman LH. Cohen's Pathways of the Pulp Expert Consult. 11th ed. Philadelphia: Elsevier Health Sciences; 2016. p. 2–32.

25. Ørstavik D, Kerekes K, Eriksen HM. The periapical index: a scoring system for radiographic assessment of apical periodontitis. Endod Dent Traumatol 1986;2:20–34.

26. European Society of Endodontology. Quality guidelines for endodontic treatment: consensus report of the European Society of Endodontology. Int Endod J 2006;39:921–30.

27. Cotti E, Campisi G, Garau V, Puddu G. A new technique for the study of periapical bone lesion; ultrasound real time imaging. Int Endod J 2002;35:148–52.

28. Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. Int Endod J 2011;44:583–609.

29. Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 2: tooth survival. Int Endod J 2011;44:610–25.

30. Sommer RF, Ostrander FD, Crowley MC, editors. Clinical Endodontics. Philadelphia: WB Saunders; 1956. p. 267–70.

31. Harrison JW, Larson WJ. The epithelized oral sinus tract. Oral Surg Oral Med Oral Pathol 1976;42:511–7.

32. Sassone LM, Fidel R, Faveri M, et al. Microbiological evaluation of primary endodontic infections in teeth with and without sinus tract. Int Endod J 2008;41:508–15.

33. Happonen RP. Periapical actinomycosis: a follow-up study of 16 surgically treated cases. Endod Dent Traumatol 1986;2:205–9.

34. Ricucci D, Siqueira Jr JF. Apical actinomycosis as a continuum of intraradicular and extraradicular infection: case report and critical review on its involvement with treatment failure. J Endod 2008;34:1124–9.

35. Siqueira JF Jr, Rôças IN, Ricucci D, Hulsmann M. Causes and management of posttreatment apical periodontitis. Br Dent J 2014;216:305–12.

36. Ricucci D, Loghin S, Gonçalves LS, et al. Histobacteriologic conditions of the apical root canal system and periapical tissues in teeth associated with sinus tracts. J Endod 2018;44:405–13.

37. Shobatake C, Miyagawa F, Fukumoto T, et al. Usefulness of ultrasonography for rapidly diagnosing cutaneous sinus tracts of dental origin. Eur J Dermatol 2014;24:683–7.

38. Kurtz AB. American institute of ultrasound in medicine. The AIUM celebrates 50 years of excellence. Available at: https://www.aium.org/aboutUs/history/articles/jum1.pdf. Accessed October 9, 2018.

39. Girish G, Jacobson JA. Ultrasound. In: Weissman BN, editor. Imaging of Arthritis and Metabolic Bone Disease. Philadelphia: Saunders; 2009. p. 89–103.

Chapter 6

Ultrasound examination for the detection of simulated periapical bone lesions in bovine mandibles: an ex vivo study

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Ultrasound examination for the detection of simulated periapical bone lesions in bovine mandibles: an *ex vivo* study

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Abstract

Musu D, Cadeddu Dessalvi C, Shemesh H, Frenda MG, Mercuro G, Cotti E. Ultrasound examination for the detection of simulated periapical bone lesions in bovine mandibles: an *ex vivo* study. *International Endodontic Journal*, **53**, 1289–1298, 2020.

Aim To evaluate the accuracy of ultrasound examination (USE) for the detection of artificial bone defects in bovine mandibles in the absence of complete erosion of the cortical bone plate and to determine the minimum cortical thickness that constitutes a barrier for ultrasound waves.

Methodology Sixty bovine mandibular anatomical blocks were harvested and uniformly distributed amongst six experimental groups. The negative control consisted of blocks with no intra-bony defects, whereas the positive control consisted of blocks with an artificial lesion of 2 mm diameter that perforated the buccal cortical bone plate. Two experimental groups comprised blocks with small (2 mm) and large (5 mm) artificial defects created under a cortical plate thinned to varying thicknesses. Two additional groups had small (2 mm) and large (5 mm) artificial defects that did not involve the cortical plate. After USE, the scans were saved and submitted to three blinded examiners. Sensitivity, specificity, predictive values and receiver-operating characteristics (ROC) were analysed. The significance of the findings ($P < 0.05$) was appraised using the chi-square statistics with the Yates correction, whilst the intra- and inter-examiner agreements were evaluated through Kappa statistics.

Results USE was associated with high sensitivity (97.3%) and negative predictive value (89%), and a perfect score for specificity and positive predictive value. The ROC curve analysis revealed an accuracy of 97.8%. The *k*-values were 0.86 and 0.89 for the first and second examinations, respectively, demonstrating very high inter-observer agreement. The intra-observer agreement was also high (*k*-value = 0.92). A significant correlation between the echographic diagnosis and the presence or absence of artificial intraosseous lesions in the anatomical blocks of bovine mandibles was observed ($P < 0.0001$).

Conclusions USE was highly accurate and reliable for the detection of artificial lesions within bovine mandibles, regardless of the thickness or presence of the cortical plate.

Keywords: apical periodontitis, bone lesions, Endodontics, ultrasound examination, ultrasound imaging.

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Introduction

Ultrasound examination (USE) or echography has been used in several fields of medicine without reports of harmful effects in humans and is widely acknowledged as a safe and cost-effective imaging technique (Duck 2001, Moore & Copel 2011). Echography has also occasionally been used for the differential diagnosis of intraosseous bone lesions in oral surgery (Dib *et al.* 1996). The use of ultrasound for the study of apical periodontitis (AP) (Cotti *et al.* 2002) resulted in the successful detection of AP. This was followed by an attempt to differentiate apical cystic lesions from granulomas (Cotti *et al.* 2003). When the results from this examination were compared to the histopathologic findings, a high accuracy in depicting the major characteristics of the lesions was demonstrated (i.e. presence or absence of vascularity within the lesion and fluid versus solid content of the lesion). Other researchers used similar protocols and confirmed these findings (Gundappa *et al.* 2006, Raghav *et al.* 2010, Sandhu *et al.* 2015). A recent systematic review (Musu *et al.* 2016) focused on the predictability of USE in the differential diagnosis of various intraosseous bone lesions of the jaws and highlighted promising results, especially in the field of Endodontics. Further studies investigating USE explored the implementation of this technique to monitor the healing of AP after orthograde (Maity *et al.* 2011) and surgical (Tikku *et al.* 2010) endodontic treatments and to assess the early response of AP to root canal treatment (Cotti *et al.* 2018).

Given the growing interest in this technique, several concerns had been raised. The first is the potential difficulty when interpreting ecographic images by untrained practitioners (Patel *et al.* 2009a); however, this limitation remains speculation (Musu *et al.* 2016). Another concern for the successful application of echography to the study of intraosseous lesions of the jaws is the potential obstacle of the thick intact cortical bone plate through which the ultrasonic beam must be transmitted (Dib *et al.* 1996, Bayrakdar *et al.* 2018). It has been argued that the prerequisite to identify a lesion within the jaws using USE is a perforated or eroded buccal cortical plate (Rajendran & Sundaresan 2007, Patel *et al.* 2009a), although this claim has not been substantiated. Conversely, thinning of the cortical plate would easily create an acoustic window for the ultrasonic waves to reach and detect the central lesions (Raghav *et al.* 2010, Ferreira *et al.* 2016), similar to conventional radiography, where minimum involvement of one of the cortical plates is needed to reveal the pathosis (Bender & Seltzer 1961). Currently, there is no standard reference for the minimum cortical bone thickness in an anatomical model to create an acoustic window that allows ultrasonic waves to enter the lesion. The aims of this *ex vivo* study were to evaluate the efficacy of USE for the detection of artificial periapical lesions within bovine mandibles in cases without complete bone erosion, to determine the minimum thickness of the cortical plates that acts as a barrier for ultrasound waves and to assess the reliability and feasibility of USE in endodontic clinical practice for the detection and differential diagnosis of AP.

Materials and methods

Ten fresh mandibles of young bovines (mean age = 2 years) were obtained. Each mandible was split sagittally at the midline. The anterior parts of the segments (containing the incisors) were excluded, and the posterior mandibles having a thicker cortical bone were used. The 20 dentulous posterior halves were each divided into three parts using a high-speed cutting band saw (MKB 752 Selekta 2; MADO, Frankfurt, Germany) to obtain three anatomical blocks per segment (mesial, middle and distal). A total of 60 specimens were thus produced. In all study groups, the cortical plate of the alveolar bone to be removed was outlined with a precision cut-off metal wheel on a rotary drill (Dremel 300; Dremel, Racine, WI, USA) at 35 000 rpm, and a sharp chisel was used to separate the outer fragment from the inner cancellous bone. The specimens were randomly allocated to six experimental groups of 10 blocks each (Fig. 1):

1. Negative control group (NCG) = no intra-bony defects were created
2. Positive control group (PCG) = one artificial lesion of 2 mm diameter perforating the cortical bone plate was created in each specimen
3. Study group 1 (SG1) = one lesion of 5 mm diameter was created in the inner bone layer, whilst the cortical plate of each sample was thinned to create different thicknesses
4. Study group 2 (SG2) = one lesion of 2 mm diameter was created in the inner bone layer with the cortical plate thinned to create different thicknesses for each specimen
5. Study group 3 (SG3) = one lesion of 5 mm diameter was created in the inner cancellous bone layer, leaving the cortical plate intact

6. Study group 4 (SG4) = the cortical plate was intact, and lesions 2 mm in diameter were performed.

Subject material preparation

The smaller experimental bone defects in PCG, SG2 and SG4 were created using a round diamond bur of 2 mm diameter (Diatech, Mt Pleasant, SC, USA) and a high-speed dental handpiece inserted from the outer surface of the buccal cortex up to the depth of the drill. In SG2 and SG4, the lesions were made in the inner cancellous bone layer once the cortical plate had been removed temporarily. The larger artificial bone defects were created using a carbide round bur of 5 mm diameter (Meisinger, Centennial, CO, USA) and a low-speed dental handpiece inserted into the inner cancellous bone. The bucco-lingual and mesio-distal diameters were verified using a digital dental calliper with an accuracy of 0.03 mm (METRICA, Milan, Italy). In the SG3 and SG4 groups, the cortical plate was left intact, whereas in the SG1 and SG2 groups, the cortical plates were thinned at specific decreasing thicknesses (from 5 to 0.5 mm with 0.5-mm intervals between adjacent blocks). The sequential reduction in thickness was achieved gradually using a sanding drum (Dremel) mounted on the rotary device and a dental model trimmer, and the results verified with a digital calliper. The procedures were performed by stabilizing the blocks and cortical

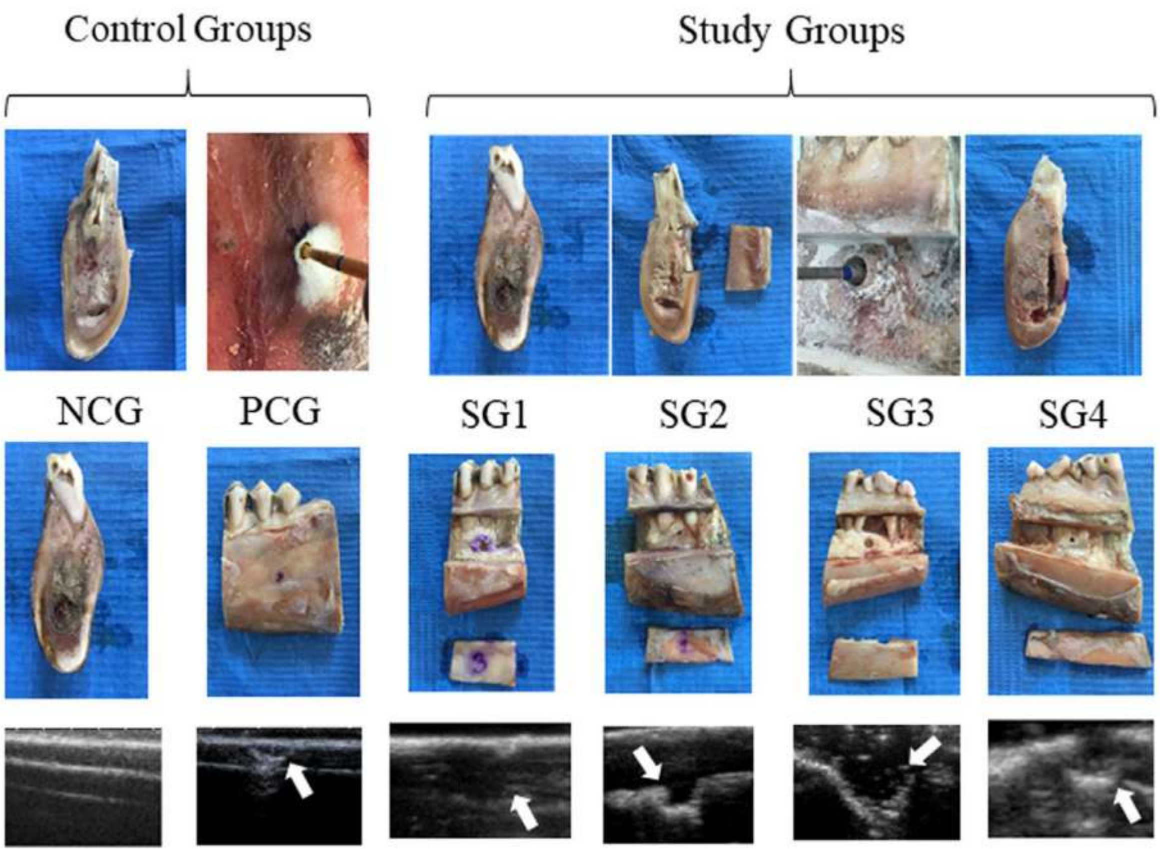

Figure 1 Schematic illustration of the anatomic blocks assigned to the different groups. In the NCG specimens, no intra-bony defects were produced, whilst in the PCG specimens lesions of 0.2 mm were produced perforating the cortical bone plate. In the experimental groups: the SG1 specimens show lesions of 0.5 mm created in the inner bone layer, and the outer cortical bone plates thinned and detached; the SG2 specimens display lesions of 0.2 mm created in the inner bone layer, and the thinned outer cortical plates detached from the block; the SG3 specimens show lesions of 0.5 mm in the inner bone layer, and the detached intact outer bone plates; the SG4 specimens reveal lesions of 0.2 mm created in the inner bone layer with detached intact cortical bone plates. The echographic images of the six experimental groups are displayed, and a B-mode scan of the artificial bone lesions for each group is shown (white arrows).

plates with a hydraulic press. The cortical plates were repositioned, and the specimens were wrapped in disposable latex bags, coded, and stored at 0 °C until USE.

Ultrasound examination

The principal investigator and an expert sonologist calibrated 10 anatomical blocks, not included in the main experiment. During the study, the anatomical blocks were filled with the ecographic gel (Aquagel; HP, Palo Alto, CA, USA) to exclude the presence of gas/air and to avoid artefacts and the creation of an acoustic barrier. The USE was performed with a Toshiba Medical-Aplio i700, (Toshiba, Tokyo, Japan) using a high-frequency linear transducer where the frequency was reduced to 4.5 Mhz to increase the penetration of the ultrasonic waves through the cortical bone. The machine was setup at the musculoskeletal standard, wherein for each sample, the sonographer varied gain and compression to enhance the accuracy of detection of the single lesion. The specimens were examined following the application of the echographic gel with the ultrasound probe placed over the repositioned cortical plate corresponding to the area of the artificial lesions. The probe was moved several times to achieve an adequate number of scans and define the bony defect. Once the lesion was located, its mesio-distal and bucco-lingual diameters were measured, and the scan was frizzed and stored in the machine computer. The images from each specimen were exported, coded and saved as TIFF files on a laptop computer (Dell Inc., Round Rock, TX, US).

Calibration and data analysis

Three observers were calibrated separately by the principal investigator using US scans of specimens not included in the experiment. The anatomical blocks for calibration comprised samples without simulated intraosseous lesions, or with lesions perforating/thinning/not involving the cortical plate. The blinded analysis was performed only after all the observers obtained at least a substantial agreement with the main examiner (Cohen's K ranging from 0.61 to 0.80). The three observers then individually assessed the 60 scans presented as TIFF images in a randomized order, organized as a PowerPoint presentation (Microsoft Office 2013; Microsoft Corporation, Redmond, WA, US) on a laptop computer. The observers were blinded to whether a bone defect was present, and whether the cortical plate was intact, thinned or perforated. All observers were asked to diagnose the presence/absence of a lesion within a scan and to circle the defect by using the computer program. The diagnosis was considered valid only when the defect was correctly identified. The same examination was repeated 4 weeks later after changing the sequence of the images, and the results of each observer were submitted and analysed. The intra- and inter-observer agreement in the visualization of the lesions was evaluated through Kappa statistics. Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were calculated to establish the validity of the technique. Furthermore, the accuracy of the procedure was visualized by the area under the receiver-operating characteristics (ROC) curve (AUC). The significance of the findings was appraised using the chi-square statistics with the Yates correction. A P value < 0.05 was considered statistically significant.

Results

The principal investigator and sonologist, using USE in real-time, could detect all the 50 artificial intraosseous lesions. The mesio-distal and bucco-lingual diameters of the defects were measured with an echographic device to confirm the diagnoses. The NCG images appeared as a cross-sectional/longitudinal echo-tomographic scanning of a mandibular area devoid of bony defects. The outer cortical plate was a highly reflective hyperechoic band, whereas the intermediate layer (medullary bone) showed an echogenic and scattered appearance until the inner cortical plate or the root surface was reached, determining the complete reflection and exhaustion of the remaining echoes (Fig. 2a). In the PCG, the 2-mm lesions perforating the cortical plate were directly accessible by the echographic probe. The scans showed the interruption of the outer bone plate as a reduction of the echogenicity overlying the bone defect, presenting as a well-defined hypoechoic area with a posterior enhancement (Fig 2b). USE also permitted the detection of the artificial bone lesions of both 2 and 5 mm diameters beneath the cortical bone plate thinned at different thicknesses in SG1 and SG2, as well as beneath the intact cortical bone plate in SG3 and SG4. In all study groups, the outer plate (independent of its thickness) could be seen as a continuous hyperechoic band, whereas the bone defects were well-defined anechoic to hypoechoic areas that interrupted the continuity of the inner alveolar bone and presented a hyperechoic posterior acoustic enhancement (Fig. 2c–f).

The mean (standard deviation [SD]) agreement between the observers for the blinded examination was 95.6 % (0.88% SD) and 96.7% (0.00% SD) during the first and second examination, respectively, after 4 weeks. The Kappa analysis showed k-values of 0.86 (0.03 SD) and 0.89 (0.00 SD) during the first and second examination, respectively, demonstrating a strong inter-observer agreement. The intra-observer agreement was very high with a mean (SD) agreement of 97.8% (0.92%) and a mean k-value of 0.92 (0.03 SD; Table 1).

The results of the sensitivity, specificity, PPV and NPV are presented in Table 2. The mean (SD) observer calculations showed a high sensitivity value of 97.3% (1.63% SD) and a high NPV of 89% (6.86% SD). These values were attributable to one false-negative result for the observers 1 and 2 during the first examination, followed by two false negatives in the second examination, and two false negatives for observer 3 in the first examination. The technique demonstrated a perfect specificity and PPV with the mean values of 100% (0.0% SD) and 100% (0.0% SD), respectively (Table 2). According to the ROC curve analysis, the overall mean value for the diagnostic accuracy of USE for the blinded observers was 97.8% (1.34% SD; Table 3).

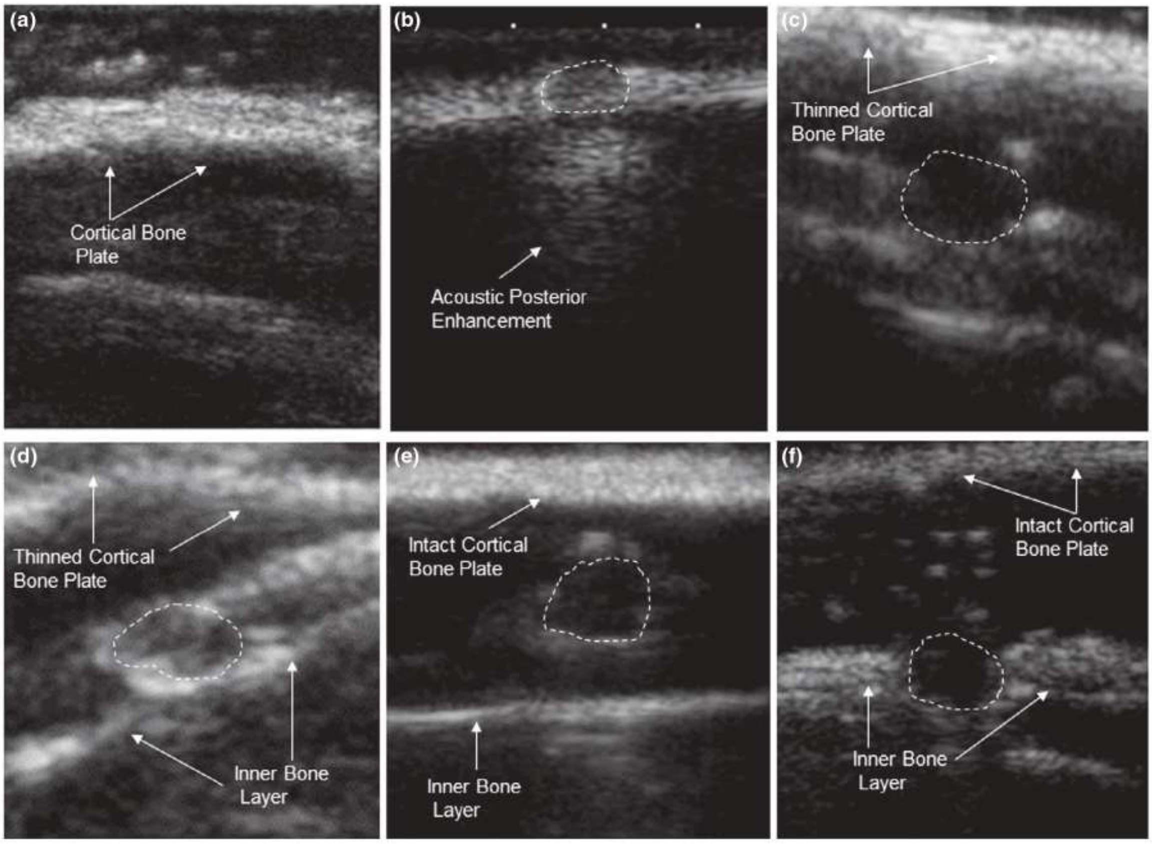

Figure 2 Echographic images of the six experimental groups: (a) NCG ultrasound scanning showing the absence of lesions and a highly reflecting cortical bone plate (arrowed); (b) PCG ultrasound image showing a hypoechoic 0.2 mm lesion (circled) perforating the cortical bone plate and producing an acoustic posterior enhancement (arrowed); (c) SG1 and (d) SG2 ultrasound scans showing the thinned outer cortical plate (arrowed) and the hypoechoic experimental lesions (circled) delimited by the inner bone layer (arrowed); (e) SG3 and (f) SG4 ultrasound scans showing the intact cortical plate (arrowed) and the hypoechoic experimental lesions (circled) delimited by the inner bone layer (arrowed).

Table 1 Percentage of agreement (%) between the observers and Kappa values for intra- and inter-observer agreement in the two different assessments

Examiner	Observer 1	Observer 2	Observer 3
Observer 1	(98.3%)[a] 0.94	(96.7%)[c] 0.89	(96.7%)[c] 0.89
Observer 2	(96.7%)[b] 0.89	(98.3%)[a] 0.94	(96.7%)[c] 0.89
Observer 3	(95%)[b] 0.84	(95%)[b] 0.84	(96.7%)[a] 0.89
	Inter-observer agreement on the 1st assessment	Inter-observer agreement on the 2nd assessment	Intra-observer agreement
Mean (SD)	95.6 % (0.88%) 0.86 (0.03)	96.7% (0.0%) 0.89 (0.0)	97.8% (0.92%) 0.92 (0.03)

SD, standard deviation.
[a]Intra-observer agreement is presented in the diagonal.
[b]Inter-observer agreement from the first assessment is presented below the diagonal.
[c]Inter-observer agreement from the second assessment is presented above the diagonal.

The chi-square statistics with the Yates correction showed a significant correlation between the echographic diagnosis of each observer and the presence/absence of artificial intraosseous lesions in the anatomical blocks ($P < 0.0001$; Table 3). No significant correlation was found between the USE diagnosis and the presence/absence of perforation of the buccal plate ($P > 0.05$), diameter of the artificial lesions ($P > 0.05$) or presence of a thinned or intact cortical bone plate ($P > 0.05$; Table 4). The average minimum thickness of the intact cortical bone plates (SG3-SG4) was 5.40 mm (0.85 SD), and 55% of the plates had >5.0 mm thickness. The average maximum thickness of the intact cortical plates was 8.32 mm (1.82 SD), and 100% of the plates had >5.0 mm thickness. The mean thickness of the cortical plates was 6.86 mm (2.04 SD).

Discussion

Despite the three-dimensional (3-D) nature of the examination, it has been reported that the major technical limitation for the use of USE in Endodontics, and Dentistry in general, lies in the hypothesis that ultrasound waves are blocked by intact cortical bone (Tikku *et al.* 2010). However, it has also been stated that an interruption in the cortical plate is not necessary to visualize a periapical lesion, since a thinning of the cortex could be sufficient to create an acoustic window for the ultrasonic waves to penetrate the bony defect (Gundappa *et al.* 2006, Patel *et al.* 2009a, Raghav *et al.* 2010). Nevertheless, such thinning has never been measured or quantified. Moreover, the authors of a previous *in vivo* study concluded that there is a significant association between the thickness of the cortical bone and the ability of ultrasound to detect the presence of periapical lesions (Tikku *et al.* 2016). However, in this report, the measures of the residual cortical plate were extrapolated by the mean thicknesses described in other studies and not directly measured (Jin *et al.* 2005, Katranji *et al.* 2007).

The present study was therefore designed to evaluate if, and how, different thicknesses of the cortical plate would influence the capability of USE to detect artificial lesions in bone, using an *ex vivo* model.

According to the results, the reading of the echographic images performed by the observers demonstrated that USE is highly reliable for the detection of the artificial central lesions. These findings are in contrast with the concerns expressed on the need of extensive clinical expertise and training to read USE scans (Rajendran & Sundaresan 2007, Patel *et al.* 2009a). Needless to say, experience and level of training have an influence on the best performance of the clinician in the interpretation of all diagnostic images (Tewary *et al.* 2011). The inter- and intra-examiner agreement in this work were optimal, and comparable to those obtained by cone beam computed tomography (CBCT) *ex vivo* human studies (Patel *et al.* 2009b, Al-Nuaimi *et al.* 2016), probably because the display of the anatomy crossed by the ultrasound waves is

Table 2 Blinded observer results and overall mean (SD) sensibility, specificity, PPV and NPV

	Sensibility		Specificity		Positive predictive value (PPV)		Negative predictive value (NPV)	
Observer	T_0	T_{4w}	T_0	T_{4w}	T_0	T_{4w}	T_0	T_{4w}
Observer 1	98%	96%	100%	100%	100%	100%	91%	83%
Observer 2	98%	96%	100%	100%	100%	100%	91%	83%
Observer 3	96%	100%	100%	100%	100%	100%	83%	100%
Mean (SD)	97.3% (1.63%)		100% (0.0%)		100% (0.0%)		89% (6.86%)	

T_0, First-time examination; T_{4W}, examination after 4 weeks.

Table 3 Blinded observer results and overall mean (SD) accuracy, ROC (AUC) and chi-square test

	Accuracy		ROC (AUC)		Chi-square		Chi-square probability	
Observer	T_0	T_{4W}	T_0	T_{4W}	T_0	T_{4W}	T_0	T_{4W}
Observer 1	98.3%	96.7%	0.99	1.0	47.109	42.188	<0.0001	<0.0001
Observer 2	98.3%	96.7%	0.99	1.0	47.109	42.188	<0.0001	<0.0001
Observer 3	96.7%	100%	1.0	1.0	42.188	62.072	<0.0001	<0.0001
Mean (SD)	97.8% (1.34%)							

ROC (AUC), Area under the ROC curve; T_0, First-time examination; T_{4W}, examination after 4 weeks.

3D as well. On the contrary, the inter- and intra-observer agreements in the diagnosis of AP with periapical radiographs, which is still considered the gold standard (Torabinejad *et al.* 2018), ranged from less than 25% to 68%, and 47% and 88%, respectively (Goldman *et al.* 1972, Goldman *et al.* 1974, Tewary *et al.* 2011) due to the two-dimensional nature of the examination.

Ultrasound imaging was 100% accurate in diagnosing the absence of intraosseous lesions (100% specificity) and, with a 100% PPV, revealed an extremely low chance (0%) to over-diagnose the presence of a diseased area, similarly to recent CBCT studies, (de Paula-Silva *et al.* 2009, Patel *et al.* 2009b, Kanagasingam *et al.* 2017b). In addition, both the data regarding USE and CBCT exhibited higher specificity and PPV than conventional and digital periapical radiographs according to the results reported by a systematic review and meta-analysis on *ex vivo* and *in vitro* studies (Leonardi Dutra *et al.* 2016). These high values of specificity and PPV can be easily explained by the fact that in the absence of intrabony lesions, and thus in the presence of sound bone, complete reflection of the ultrasound waves occurred. In addition, USE revealed high values of sensitivity (97.3%) and NPV (89%) in diagnosing the incidence of bone lesions, nevertheless the presence of false negatives determined a risk of missing the disease in 11% of the cases. Despite this, the USE sensitivity and NPV had higher values than those of digital and conventional periapical radiographs (Stavropoulos & Wenzel 2007, Leonardi Dutra *et al.* 2016, Kanagasingam *et al.* 2017a). Unfortunately, these values could not be compared to those from other USE studies since experiments with a similar design are not available.

The accuracy of USE in the detection of artificial periapical lesions (97.8%) exhibited values higher than those reported for periapical radiographs in other *ex vivo* studies (Stavropoulos & Wenzel 2007, Paula-Silva *et al.* 2009, Leonardi Dutra *et al.* 2016, Kanagasingam *et al.* 2017a) comparable to those of CBCT (Paula-Silva *et al.* 2009, Patel *et al.* 2009b, Liang *et al.* 2014, Leonardi Dutra *et al.* 2016, Kanagasingam *et al.* 2017b). Most importantly, the ability to detect the lesions within the anatomical blocks using echography was independent of the diameter of the lesions, presence/absence of perforation in the cortical plate and thickness of the bony barrier (Table 4). The easiest way to explain this finding is to consider that, as mentioned above, US examination is a three-dimensional image obtained by moving the probe on the sector plane whilst performing the examination (Auer & Van Velthoven 1990).

A previous attempt to validate the detection of intraosseous lesions using USE with respect to the thickness of the bone plate (Adibi *et al.* 2015) led to a cut-off measure of 1.1 mm cortical thickness to mask an intra-bony defect. The model for this experiment was limited because it consisted of a flat bone surface where the lesions were created on the opposite side of the cortex, thus allowing air to enter the artificial defects from behind. Ultrasound waves in the presence of an interface between bone and air can undergo total reflection without exposing the underlying lesion (Auer & Van Velthoven 1990, Abu-Zidan *et al.* 2011). This phenomenon does not occur *in vivo*, and to avoid the interference of the air and simulate a clinical setting in this study, the bony defects and empty spaces between the removed cortical plate and remaining anatomical block were filled with echographic gel.

The present work is the first *ex vivo* experiment using an anatomical model of artificial periapical lesions to evaluate the capability and feasibility of USE in the detection of central lesions, of different diameters, within the jaws having various thicknesses of cortical plate involvement. A negative control group (NCG) was included based on the fact that a diagnostic test should be able to detect the disease where it is present and to exclude healthy subjects

Table 4 Statistical correlation between the detectability of the lesions and the different variables

Variable	Presence of perforation Chi-square (*P* value)		Diameter of the lesions Chi-square (*P* value)		Thinning of the cortical plate Chi-square (*P* value)	
	T_0	T_{4W}	T_0	T_{4W}	T_0	T_{4W}
Observer 1	0.255 ($P = 0.6135$)*	0.521 ($P = 0.4705$)*	0.043 ($P = 0.8366$)*	1.063 ($P = 0.3024$)*	1.026 ($P = 0.3112$)*	0.0 ($P = 1.0$)*
Observer 2	0.574 ($P = 0.4487$)*	0.033 ($P = 0.8568$)*	0.680 ($P = 0.4095$)*	0.087 ($P = 0.7683$)*	0.0 ($P = 1.0$)*	1.026 ($P = 0.3112$)*
Observer 3	0.521 ($P = 0.4705$)*	0.0 ($P = 1.0$)*	1.063 ($P = 0.3024$)*	0.0 ($P = 1.0$)*	0.0 ($P = 1.0$)*	0.0 ($P = 1.0$)*

T_0, First-time examination; T_{4W}, examination after 4 weeks.
*Not significant, $P < 0.05$

and thus establish an appropriate health reference standard (Pope *et al.* 2014, Kanagasingam *et al.* 2017b). Furthermore, the second group of specimens (PCG) served as positive control group since the artificial bone lesions of 0.20 mm perforating the cortical bone plate were directly accessible to the echographic probe without any acoustic barrier.

In this study, 2 mm was established as the smaller diameter for the artificial lesions (groups SG2 and SG4) whilst 5 mm was the larger diameter established (groups SG1 and SG3). This was based since the early research comparing the accuracy of CBCT in the detection of AP with histopathological or surgical references rarely considered lesions having a diameter larger than 2 mm (de Paula-Silva *et al.* 2009, Patel *et al.* 2009b, Tsai *et al.* 2012). In contrast, periapical lesions of 5.0 mm in diameter are considered easily detectable by conventional, 3-D and sonographic imaging (Barbat & Messer 1998, Liang *et al.* 2014, Adibi *et al.* 2015, Nardi *et al.* 2017). An important clinical implication from these results is that both small and large bone defects can be measured and located with USE at this experimental setting, also in the presence of an intact buccal bone plate. Furthermore, the outcome of a treatment could be assessed by monitoring the reduction in size of a lesion in the presence of new bone formation during the healing process.

A limitation of this study was that it was not possible to use human specimens. Bovine fresh mandibles were selected because of their high mineral density, making the examination more difficult than that of human samples (Alves *et al.* 1996). Additionally, the choice of the posterior mandibles was dictated by the necessity to produce anatomical blocks with a cortical thickness equal or greater than that of human samples. Anatomical studies have revealed that the thickness of the human buccal cortical bone in the posterior mandible has a mean maximum value ranging from 2.32-3.18 mm, with the greatest registered thickness of 5.25 mm in the apical region of the second molar (Baumgaertel & Hans 2009, Al-Jandan *et al.* 2013). These thicknesses were considerably lower than the measurements registered in the present study. Another limitation of the present *ex vivo* model was the unavoidable presence of artefacts produced during the harvesting of the anatomical blocks. The separation of the cortical plate from the cancellous bone produced irregularities on both surfaces at the cutting line that were visible on the echotomograms, mimicking small lesions. Another artefact was caused by the interposition of air when the cortical plates were repositioned on their anatomical blocks, creating an acoustic shadow. Lastly, the absence of soft tissues associated with the anatomical blocks could have facilitated the detection of the artificial lesions. Although these results seem promising, further studies comparing USE with CBCT should be conducted on humans to investigate lesions of smaller diameters. Studies performed with different ultrasound devices could be also useful to standardize the technique.

Conclusion

The present *ex vivo* experiment demonstrated that artificial bony lesions in bovine mandible bones were detectable with USE in a predictable way and were independent of the diameter, thickness and presence/absence of perforation in the buccal cortical plate. USE could be used to complement the 3-D imaging of AP.

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Conflict of interest

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References

Abu-Zidan FM, Hefny AF, Corr P (2011) Clinical ultrasound physics. *Journal of Emergencies Trauma and Shock* **4**, 501–3.

Adibi S, Shakibafard A, Sarvestani ZK, Saadat N, Khojastepour L (2015) Effect of cortical bone thickness on detection of intraosseous lesions by ultrasonography. *Radiology Research and Practice* **2015**, 1–6.

Al-Jandan BA, Al-Sulaiman AA, Marei HF, Syed FA, Almana M (2013) Thickness of buccal bone in the mandible and its clinical significance in mono-cortical screws placement. A CBCT analysis. *International Journal of Oral and Maxillofacial Surgery* **42**, 77–8.

Al-Nuaimi N, Patel S, Foschi F, Mannocci F (2016) The detection of simulated periapical lesions in human dry mandibles with cone beam computed tomography – a dose reduction study. *International Endodontic Journal* **49**, 1095–104.

Alves JM, Xu W, Lin D, Siffert RS, Ryaby JT, Kaufman JJ (1996) Ultrasonic assessment of human and bovine trabecular bone: a comparison study. *IEEE Transactions on Biomedical Engineering* **43**, 249–58.

Auer LM, Van Velthoven V (1990) *Intraoperative Ultrasound Imaging in Neurosurgery*. Berlin: Springer Verlag, pp 1–11.

Barbat J, Messer HH (1998) Detectability of artificial periapical lesions using direct digital and conventional radiography. *Journal of Endodontics* **24**, 837–42.

Baumgaertel S, Hans MG (2009) Buccal cortical bone thickness for mini-implant placement. *American Journal of Orthodontics and Dentofacial Orthopedics* **136**, 230–5.

Bayrakdar IS, Yilmaz AB, Caglayan F, Ertas U, Gundogdu C, Gumussoy I (2018) Cone beam computed tomography and ultrasonography imaging of benign intraosseous jaw lesion: a prospective radiopathological study. *Clinical Oral Investigations* **22**, 1531–9.

Bender IB, Seltzer S (1961) Roentgenographic and direct observation of experimental lesions in bone. *Journal of the American Dental Association* **87**, 708–16.

Cotti E, Campisi G, Garau V, Puddu G (2002) A new technique for the study of periapical bone lesion; ultrasound real time imaging. *International Endodontic Journal* **35**, 148–52.

Cotti E, Campisi G, Ambu R, Dettori C (2003) Ultrasound real-time imaging in the differential diagnosis of periapical lesions. *International Endodontic Journal* **36**, 556–63.

Cotti E, Esposito SA, Musu D, Campisi G, Shemesh H (2018) Ultrasound examination with color power Doppler to assess the early response of apical periodontitis to the endodontic treatment. *Clinical Oral Investigations* **22**, 131–40.

de Paula-Silva FW, Wu MK, Leonardo MR, da Silva LA, Wesselink PR (2009) Accuracy of periapical radiography and cone-beam computed tomography scans in diagnosing apical periodontitis using histopathological findings as a gold standard. *Journal of Endodontics* **35**, 1009–12.

Dib LL, Curi MM, Chammas MC, Pinto DC, Torloni H (1996) Ultrasonography evaluation of bone lesions of the jaw. *Oral Surgery Oral Medicine Oral Pathology* **82**, 351–7.

Duck FA (2001) Recent epidemiology papers: European committee for medical ultrasound safety. *Ultraschall in der Medizin* **13**, 227–31.

Ferreira TLD, Costa ALF, Tucunduva MJA, Tucunduva-Neto RR, Shinohara EH, de Freitas CF (2016) Ultrasound evaluation of intra-osseus cavity: a preliminary study in pig mandibles. *Journal of Oral Biology and Craniofacial Research* **6**, S14–17.

Goldman M, Pearson AH, Darzenta N (1972) Endodontic success – who's reading the radiograph? *Oral Surgery Oral Medicine Oral Pathology* **33**, 432–7.

Goldman M, Pearson AH, Darzenta N (1974) Reliability of radiographic interpretations. *Oral Surgery Oral Medicine Oral Pathology* **38**, 287–93.

Gundappa M, Ng SY, Whaites EJ (2006) Comparison of ultrasound, digital and conventional radiography in differentiating periapical lesions. *Dentomaxillofacial Radiology* **35**, 326–33.

Jin GC, Kim KD, Roh BD, Lee CY, Lee SJ (2005) Buccal bone plate thickness of the Asian people. *Journal of Endodontics* **31**, 430–4.

Kanagasingam S, Hussaini HM, Soo I, Baharin S, Ashar A, Patel S (2017a) Accuracy of single and parallax film and digital periapical radiographs in diagnosing apical periodontitis - a cadaver study. *International Endodontic Journal* **50**, 427–36.

Kanagasingam S, Lim CX, Yong CP, Mannocci F, Patel S (2017b) Diagnostic accuracy of periapical radiography and cone beam computed tomography in detecting apical periodontitis using histopathological findings as a reference standard. *International Endodontic Journal* **50**, 417–26.

Katranji A, Misch K, Wang HL (2007) Cortical bone thickness in dentate and edentulous human cadavers. *Journal of Periodontology* **78**, 874–8.

Leonardi Dutra K, Haas L, Porporatti AL *et al.* (2016) Diagnostic accuracy of cone-beam computed tomography and conventional radiography on apical periodontitis: a systematic review and meta-analysis. *Journal of Endodontics* **42**, 356–64.

Liang YH, Jiang L, Gao XJ, Shemesh H, Wesselink PR, Wu MK (2014) Detection and measurement of artificial periapical lesions by cone-beam computed tomography. *International Endodontic Journal* **47**, 332–8.

Maity I, Kumari A, Shukla AK, Usha HL, Naveen DN (2011) Monitoring of healing by ultrasound with color power doppler after root canal treatment of maxillary anterior teeth with periapical lesions. *Journal of Conservative Dentistry* **14**, 252–7.

Moore CL, Copel JA (2011) Point-of-care ultrasonography. *New England Journal of Medicine* **364**, 749–57.

Musu D, Rossi-Fedele G, Campisi G, Cotti E (2016) Ultrasonography in the diagnosis of bone lesions of the jaws: a systematic review of the literature. *Oral Medicine Oral Surgery Oral Pathology Oral Radiology* **122**, e19–29.

Nardi C, Calistri L, Pradella S, Desideri I, Lorini C, Colagrande S (2017) Accuracy of orthopantomography for apical periodontitis without endodontic treatment. *Journal of Endodontics* **43**, 1640–6.

Patel S, Dawood A, Whaites E, Pitt Ford T (2009a) New dimensions in endodontic imaging: part 1. Conventional and alternative radiographic systems. *International Endodontic Journal* **42**, 447–62.

Patel S, Dawood A, Mannocci F, Wilson R, Pitt Ford T (2009b) Detection of periapical bone defects in human jaws using cone beam computed tomography and intraoral radiography. *International Endododontic Journal* **42**, 507–15.

Pope O, Sathorn C, Parashos P (2014) A comparative investigation of cone-beam computed tomography and periapical radiography in the diagnosis of a healthy periapex. *Journal of Endodontics* **40**, 360–5.

Raghav N, Reddy SS, Giridhar AG *et al.* (2010) Comparison of the efficacy of conventional radiography, digital radiography and ultrasound in diagnosis of periapical lesions. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontology* **110**, 379–85.

Rajendran N, Sundaresan B (2007) Efficacy of Ultrasound and Color Power Doppler as a monitoring tool in the healing of endodontic periapical lesions. *Journal of Endodontics* **33**, 181–6.

Sandhu SS, Singh S, Arora S, Sandhu AK, Dhingra R (2015) Comparative evaluation of advanced and conventional diagnostic aids for endodontic management of periapical lesions: an in vivo study. *Journal of Clinical and Diagnostic Research* **9**, 1–4.

Stavropoulos A, Wenzel A (2007) Accuracy of cone beam dental CT, intraoral digital and conventional film radiography for the detection of periapical lesions: an ex vivo study in pig jaws. *Clinical Oral Investigations* **11**, 101–6.

Tewary S, Luzzo J, Hartwell G (2011) Endodontic radiography: who is reading the digital radiograph? *Journal of Endodontics* **37**, 919–21.

Tikku AP, Kumar S, Loomba K, Chandra A, Verma P, Aggarwal R (2010) Use of ultrasound, color Doppler imaging and radiography to monitor periapical healing after endodontic surgery. *Journal of Oral Science* **52**, 411–6.

Tikku AP, Bharti R, Sharma N, Chandra A, Kumar A, Kumar S (2016) Role of ultrasound and color doppler in diagnosis of periapical lesions of endodontic origin at varying bone thickness. *Journal of Conservative Dentistry* **19**, 147–51.

Torabinejad M, Rice DD, Maktabi O, Oyoyo U, Abramovitch K (2018) Prevalence and size of periapical radiolucencies using cone-beam computed tomography in teeth without apparent intraoral radiographic lesions: a new periapical index with a clinical recommendation. *Journal of Endodontics* **44**, 389–94.

Tsai P, Torabinejad M, Rice D, Azevedo B (2012) Accuracy of cone-beam computed tomography and periapical radiography in detecting small apical periodontitis. *Journal of Endodontics* **38**, 965–70.

Chapter 7

The effectiveness of ultrasound examination to assess the healing process of bone lesions of the jaws: a systematic review

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ORIGINAL ARTICLE

The effectiveness of ultrasound examination to assess the healing process of bone lesions of the jaws: a systematic review

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Abstract

Objective To assess the potential of ultrasound examination (USE) as an adjunctive method to conventional radiology to evaluate the healing processes occurring after the treatment of bone lesions of the jaws. The research question was: what is the effectiveness of USE to evaluate the healing of intra-osseous bone lesions when compared to radiological or clinical examination?

Materials and methods Six databases (PubMed, the Web of Science, Scopus, Embase, the Cochrane Library, and Best Evidence) were searched from their inception (PROSPERO CRD42019134482). A quality assessment was performed combining the Downs and Black tool and the Newcastle-Ottawa scale. The risk of bias was calculated using the Cochrane collaboration tool to assess the risk of bias.

Results A total of 4404 records were screened, and 7 studies meeting the inclusion criteria were included in the systematic review. USE allowed to evaluate the healing of jaw bone lesions by assessing their reduction in size, the increase in echogenicity of the affected area, and the progressive decrease\disappearance of the vascular flow signal within the lesions.

Conclusions USE implemented with color power Doppler is an advanced imaging technique feasible to monitor the early and long-term response of the intra-osseous lesions of the jaws to both surgical and nonsurgical treatment.

Clinical relevance This systematic review brought evidence that USE can constitute a safe alternative imaging technique in the dental clinical practice for the management of central lesions of the maxillary bones.

Keywords Ultrasound examination · Systematic review · Central bone lesions · Healing · Apical periodontitis

Introduction

Notwithstanding the variety of different radiolucent lesions that can occur in the peri-radicular area of teeth in the maxillary bones, most of them are inflammatory in origin [1]. Moreover, despite the increasing accuracy of cone-beam computed tomography (CBCT) in detecting apical periodontitis (AP) [2], the clinical signs and symptoms in conjunction with conventional intraoral radiographs remain the gold standard for the diagnosis, treatment, and follow-up of AP [3–5]. The assessment of AP through intraoral radiographs is usually based on the detection of a radiolucent area, representative of the level of destruction of bone architecture and its mineral loss caused by the inflammation of the periapical tissues as a consequence of the endodontic infection [6–8]. However, the term "apical periodontitis" is used to describe all the dynamic host responses to the infection of the root canal and their different histological features. Additionally, conventional radiology is not able to characterize these variables [9, 10]. This consideration becomes even more important when following up the AP response to the root canal treatment. In some cases, there is no radiological evidence of complete healing (i.e., a sound periodontal ligament space) even with the remission of signs and symptoms [4]; moreover, in extensive lesions, the outcome of root canal treatment may remain uncertain as there are no strict radiological criteria to differentiate apical scar tissue from AP [4, 11, 12]. Ultrasound examination (USE), also called ultrasonography or echography, is a real-time

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imaging modality based on the propagation of ultrasound waves towards the organs and tissues. While penetrating through the tissues, the ultrasound waves are partially transmitted more deeply within the body or reflected back to the probe as echoes. Traditionally, in the brightness modality (B-mode), the echoes are converted into light spots on a screen generating a grayscale 2D image which is a cross-sectional real-time image of the imaged tissue [13]. Since its introduction in endodontics, USE has proved to obtain images of AP and to collect information related to its content [14]. In further studies, USE, with the addition of the color and power Doppler (CPD), was applied to differentiate between granulomatous and cystic apical lesions using histopathology as a reference standard [15, 16]. Later studies have employed the same USE criteria to differentiate AP from other intra-osseous lesions of the jaws showing promising results [17]. The rationale behind the use of echography to assess the healing of intra-osseous bone lesions is that, by gaining information on the content of the affected area (tissues architecture, vascularity, and mineralization), the clinician would be aware of the biological phenomena involving the specific anatomical site following treatment. This systematic review aims to collect the scientific literature in order to assess the USE potential as an adjunctive method to traditional radiology to evaluate the healing processes occurring after treatment of the jaws bone lesions with special emphasis on AP.

Materials and methods

Protocol and registration

The present systematic review was performed following the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines [18]. The protocol was produced and registered in PROSPERO with the Centre for Reviews and Dissemination at the University of York under code CRD42019134482.

Information sources and search

The following electronic databases were investigated: PubMed's Medline, Embase, Scopus, and the Web of Science. The research was implemented with a subsequent additional search on the Cochrane Library and Best Evidence databases. The research strategy comprised the following keywords coupled with Boolean operators and organized as the following research algorithm for the databases search (Supplementary Table 1):

(Ultrasound Imaging OR Echography OR Ultrasonography) AND (Healing) AND (Apical Periodontitis OR Periapical OR Jaw)

The references of the studies selected for the review were screened manually in order to detect other relevant papers. In addition, gray literature was searched through appropriate databases and registers.

The search within the databases was conducted independently by two of the reviewers (D.M. and M.B.) from July 15, 2019, to August 15, 2019.

Eligibility criteria

The review question was formulated as a PICO (P, patients with jaw bone lesions\apical periodontitis; I, ultrasound examination; C, traditional radiology and\or clinical examination; O, effectiveness) in the following way: What is the effectiveness of USE to evaluate the healing of the affected area after treatment, when compared to the radiological and clinical examinations in patients with maxillary bone lesions?

The inclusion criteria were as follows:

1) Studies assessing the healing of central jaw lesions with the ultrasound imaging technique.
2) No restrictions for language or date were applied to the research.
3) Studies with human participants.

The exclusion criteria were the following:

1) Studies using ultrasounds for other purposes than monitoring the healing of lesions in the maxillary bones
2) In vitro, ex vivo, and animal studies
3) Reviews, conference papers, or letters to the editor

Study selection

In the first phase, two calibrated reviewers (D.M. and M.B.) eliminated the duplicates from the database search by using the EndNote basic software (Thompson Reuters, New York, NY). In the second phase, the two reviewers screened the records according to the titles and abstracts to exclude studies not meeting the eligibility criteria. Those papers included from the previous steps were then assessed independently of their full text. In case of disagreement between the two reviewers, a discussion was carried out in order to reach a mutual agreement. When a consensus was not reached between the two reviewers, a third trained researcher (E.C.) was involved in the discussion in order to reach a definite agreement.

Data collection process and data items

A data collection sheet was created to gather all the relevant characteristics of the studies (Table 1). The specific items extracted and organized in the table were the following: (1) author

Table 1 Characteristics of the included studies

Author year	Sample size	N° teeth	Age mean (range)	Type of teeth	Treatment	US machine	Examiner	Type of probe and position	Frequency (MHz)	Preoperative USE and diagnosis	Parameters	Follow-up USE	Comparison	Overall (max) follow-up	Outcome
Rajendran & Sundaresan (2007)	5 (4F; 1M)	5	29.6 (27–37)	Upper anterior	RCT	ALOKA Prosound-S-SD 5500	Radiologist	22-mm endocavitary Intra-orally	5–10	Yes-no	Echogenicity Size Vascularity	6 months	Clinical and radiographic	6 months	+
Aggarwal & Singla (2010)	3 (1F; 2M)	6	27.3 (23–34)	Upper anterior	RCT	LOGIQ-500 PRO	-/-	High definition multifrequency –/–	10	Yes-yes	Echogenicity	30–36 months	Clinical and radiographic (CBCT)	36 months	+
Tikku et al. (2010)	15 (-\-)	-/-	-\- (15–40)	Upper and lower anterior	Surgery	Toshiba Nemio Model SSA-550A	Radiologist	High-frequency and resolution linear Extra-orally	7–9	Yes-no	Echogenicity Size Vascularity	1 week 6 months	Radiographic	6 months	+
Maity et al. (2011)	10 (-\-)	17	-/-	Upper anterior	RCT	GE Voluson PRO 730	Radiologist	Intracavitary multifrequency Intra-orally	8–12	Yes-yes	Echogenicity Size Vascularity	6 weeks 3, 6 months	Clinical and radiographic	6 months	+
Curvers et al. (2017)	8 (4F; 4M)	8	47.6 (25–62)	Upper and lower posterior and anterior	Surgery	Flex Focus 800	Endodontist	High definition, frequency linear Extra-orally	12	Yes-no	Echogenicity Size	1 week 1, 2, 3, 6 months	–/–	6 months	+
Cotti et al. (2018)	21 (12F; 9M)	26	38.9 (–\-)	Upper and lower posterior and anterior	RCT and Surgery	Siemens Elegra Toshiba Aplio XG	Radiologist	Regular small-size, linear, high definition Extra- and intra-orally	7–9 8–12	Yes-yes	Echogenicity Size Vascularity	1, 4 weeks	Clinical and radiographic	7 years	+
Zainedeen et al. (2018)	20 (-\-)	-/-	(11–50)	Upper and lower posterior and anterior	Surgery	HS-4000	Radiologist	-/-	-/-	Yes-yes	Echogenicity Size Vascularity	1 week 6 months	Radiographic (CBCT)	6 months	+

and year of publication, (2) sample characteristics (number of patients and male\female ratio), (3) number of teeth involved, (4) population age (range and mean), (5) anatomical position of the lesions with respect to the teeth (anterior\posterior, upper\lower), (6) type of treatment performed (orthograde or surgical root canal treatment), (7) model of ultrasound apparatus, (8) specialization of the examiner who performed USE, (9) type of probe and position of the probe on the patient (intra- vs extra-oral), (10) frequency of the ultrasounds expressed in megahertz (MHz), (11) preoperative USE (yes\no) and preoperative ultrasound diagnosis, (12) ultrasound outcome parameters (echogenicity\size\vascularity), (13) follow-up with USE, (14) overall follow-up time, (15) term of comparison, (16) outcome on the effectiveness of USE in monitoring the healing of lesions in the maxillary bones (+\-). Each reviewer filled the table independently, and then the data selected by each reviewer were confirmed and cross-checked by the other investigators. Missing or unclear data from the included studies were requested by contacting the corresponding author.

Quality assessment in individual studies

Two calibrated reviewers (D.M. and M.B.) assessed the methodology and quality of the eligible studies through relevant quality appraisal tools for non-randomized clinical studies. The Newcastle-Ottawa scale and the Downs and Black tool were used in conjunction [19]. The quality appraisal checklists were used to elaborate a scoring system, where each item was scored as 0 if the item was not fulfilled, 0.5 if partially fulfilled, or 1 if it was completely fulfilled. Studies were considered of low quality (score ≤ 12), moderate quality (score between 12 and 24), and high quality (score ≥ 24) [20–22].

Summary measures

The diagnostic ability of USE to predict and monitor the healing of the affected area following treatment of intra-osseous bone lesions, when compared to other clinical and radiological observations, was considered the main outcome.

Risk of bias across studies

The risk of bias of the included studies was assessed using the Cochrane collaboration' tool [19] by two calibrated reviewers (D.M. and M.B.). The tool items were scored as 1 if the item was considered as fully fulfilled, as 0 if the item was clearly unfulfilled, and as 0.5 if the item was unclearly or only partially fulfilled. Studies with a score ≤ 2.3 were considered as having a high risk of bias, with a score between 2.4 and 4.6 of moderate risk of bias, whereas studies with a score ≥ 4.7 were considered as having a low risk of bias.

Synthesis of the results and additional analyses

A qualitative synthesis of the results was performed based on the data collected from the included studies. Such studies were analyzed in two groups to decrease the heterogenicity of the findings: those studies evaluating the healing of bone lesions by USE following root canal therapy (RCT) or those following surgical treatment. A meta-analysis would be additionally performed, depending on the data available.

Results

The papers recorded were 3881. The records identified through Embase, Scopus, PubMed, and Web of Science were 134, 77, 3650, and 19, respectively. The screening process was performed through the software EndNote (X7 version) (Thompson Reuters, CA, USA) on August 15, 2019. In phase 2, the duplicates removed from Embase, Scopus, PubMed, and Web of Science were 27, 22, 544, and 2, respectively. The authors checked 1595 titles, and the final selection was made based on the titles and abstracts independently evaluated by two investigators (D.M and M.B). A subsequent additional research on the Cochrane Library and Best Evidence produced 524 records, 420 of which were duplicates and the remaining 104 were excluded since they did not meet the eligibility criteria. According to the inclusion criteria, 7 full text articles were included from the electronic search, while 1 full text was included [23] from a complementary manual search. The latter was then excluded [23] because of the presence of duplicated cases from the same author in the 2011study [24] (Fig. 1). Possible disagreements were solved involving the third reviewer (E.C.), an expert in endodontology and oral radiology. All the 7 articles included in the qualitative synthesis were non-randomized clinical studies which monitored healing of intra-osseous lesions of the jaws after surgical and nonsurgical treatment in humans with USE [23–29]. The sample sizes ranged from three [25] to 21 patients [26] comprising 5 [27] to 26 teeth [26], and there was female predilection.

Quality assessment

Two reviewers (D.M. and M.B) completed the critical appraisal of the studies combining the two checklists with the "Downs and Black scale" by analyzing the study quality, external validity, study bias, confounding and selection bias, and power of the study, while the New Castle-Ottawa scale assessed three domains: selection of study groups, comparability of groups, and ascertainment of exposure/outcome. Three studies showed high quality [26, 28, 29] and four studies showed moderate quality [24, 25,

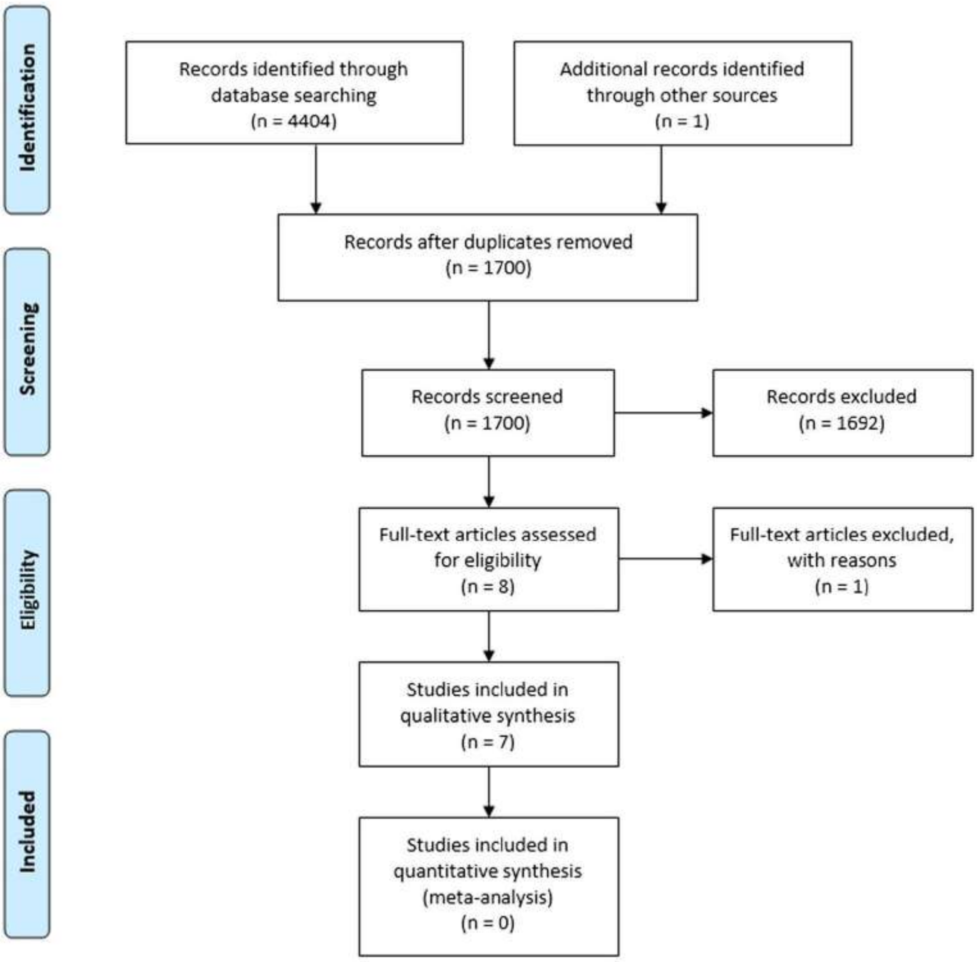

Fig. 1 PRISMA flowchart used for the study selection

27, 30] (Fig. 2). Considering the lack of studies investigating this topic and the high to moderate quality of the relevant studies, all 7 non-randomized clinical studies were included to obtain the best and most comprehensive evidence available.

Results of the individual studies

The data collected from each study are reported in Table 1. The lesions were more often documented in the anterior maxilla [24, 25, 27, 28], followed by anterior mandible [28], and

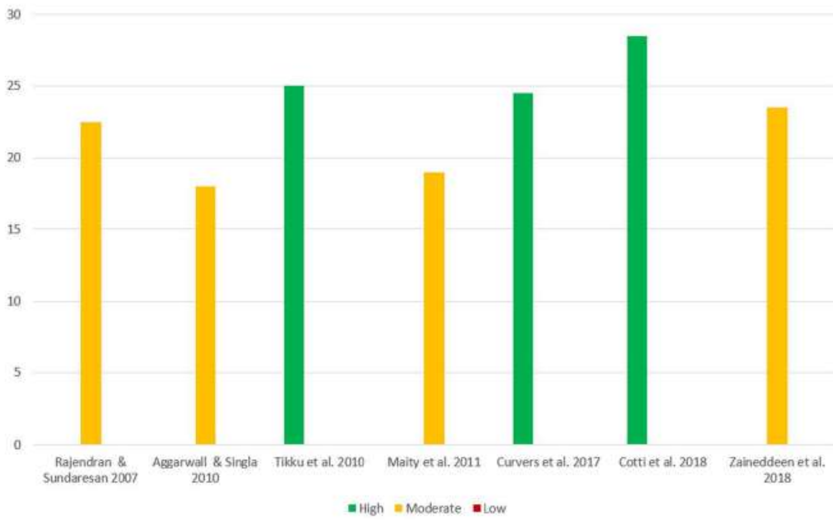

Fig. 2 Quality assessment of the included studies following the Newcastle-Ottawa scale and the Downs and Black tool

mixed locations [26, 29, 30]. Six investigations were performed on endodontic lesions and one [30] on two dysembriogenetic cysts. Healing was evaluated following clinical RCT in [24, 25, 27], or surgery [28–30], while one research combined surgical and nonsurgical cases were followed-up [26]. The USE was performed by an expert radiologist [23, 25–27, 29], a trained endodontist [29], or not specified [25]. Different ultrasound devices were used. During the exams, the probe was positioned intra-orally [24, 27], extra-orally [28, 29], either way, depending on the case [26], or not specified [25, 30]. The frequency of the ultrasounds (high frequency, ≥ 5 MHz) was described in 6 articles [23–27]. All studies performed an initial USE, but only 4 [23–25, 29] presented a preoperative echographic diagnosis. The parameters analyzed to monitor healing of the lesions were echogenicity [23–29], size [23, 25–29], and vascularity [23, 25–27, 29] of the areas. The timing of the echographic follow-up varied from 1 week [25, 27–29] to 36 months [25]. One author relied only on USE to assess healing of bone lesions [29], while the other studies confirmed healing with both radiological findings and clinical parameters [23–26], conventional radiology [24–28], or CBCT [25, 30].

Synthesis of the results

The echographic findings were clustered into two categories according to the treatment performed:

1. Root canal treatment. Ultrasound examination allows to evaluate the healing of AP following RCT by predictably showing: the reduction in size of the lesions [24, 26, 27], the increase in echogenicity, using a grayscale standard reference in B-mode [23–26], and the progressive decrease\disappearance of the vascular signal of the lesions at both color and power Doppler [24, 26, 27] when compared to clinical and radiographic findings.
2. Surgical treatment. Ultrasound examination can be considered effective to evaluate healing of the maxillary bones, after surgical excision of the lesions [26, 28–30]. Healing after the enucleation of the lesion was described within the USE scans as follows: initial increase in size [28, 29] and vascularity of the bone cavities [26, 28, 30], progressive reduction of the areas [27–29], gradual increase in echogenicity in B-mode (anechoic to hyperechoic) [27–29], and progressive decrease of the vascular signal, until disappearance, on color Doppler [28, 30] or with the color power Doppler [26].

Risk of bias

Based on the Cochrane Collaboration's tool for assessing the risk of bias [19], the authors judged each article as low, moderate, and high bias risk. Figure 3 summarizes and documents the limitations in the studies which were categorized by two independent and calibrated reviewers (D.M. and M.B.) who resolved their disagreement by discussion. Most researches showed a low risk of bias, while two studies [24, 25] showed a moderate risk of bias.

Additional analysis

The study characteristics and the data collected from the articles were not homogeneous in terms of exposures and outcomes and could not be included in a meta-analysis.

Discussion

This systematic review aimed to investigate the best available evidence on the use of USE in the assessment of maxillary bone lesions healing. The role of USE in monitoring bone healing of the jaws has been previously described to evaluate the surgical wound in the distraction osteogenesis of the mandible [30–35], a procedure requiring the precise determination of bone regeneration for a safe removal of the distraction device [31, 32, 35]. While earlier studies demonstrated USE to be reliable in the measurement of the corticotomy gap and in the detection of new bone apposition [35, 36], more recent clinical reports proved that USE can be equally adequate in the measurement of the osteotomic gap [31] and more accurate in the evaluation of the callus maturity [31, 32] with respect to traditional radiology. In the healing model presented for the distraction of the mandible, the anechoic gap of the surgical wound is first filled with echogenic material (new bone matrix) [34], followed by increasing echogenic areas after 1 week (bony islands), and by a hyperechoic barrier (outer cortical bone) at the completion of mineralization [31, 32]. The results of the present systematic review confirm that grayscale USE is an efficient tool for the early assessment and the long-term monitoring of bone healing after treatment of the intra-osseous lesions of the jaws. New data on the vascular response to treatment of the diseased site were also collected and analyzed in this work.

In the four studies conducted on surgical cases [26, 28–30], all lesions were completely removed. Consequently, USE evaluated the healing of the residual bone defects. The post-surgical healing process described with USE is characterized by the progressive decrease in the defect size of the areas, as well as by the reduction\disappearance of the CPD vascular signal. However, a temporary increase of the vascular signal may appear in 1-week follow-up [26, 28, 30]. This finding may be explained by the inflammatory response generated after the surgical trauma, essential for the removal of the surgical debris and the beginning of the repair pathways [26, 28, 30, 37]. The vascular signal is then reduced after 4 weeks [26],

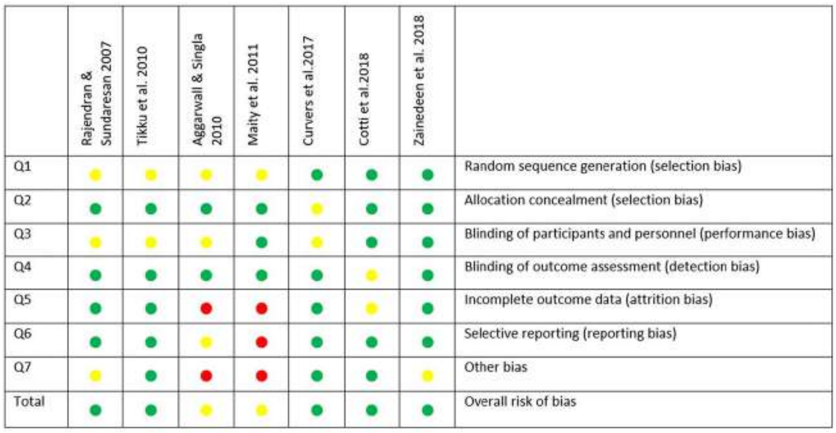

Fig. 3 Risk of bias of the included studies following the Cochrane collaboration' tool for assessing the risk of bias

Yes: ● = score 1, No: ● = score 0, partial or unclear: ● = score 0.5.

with the extinguishing of the acute inflammation phase and the increase of bone deposition [29, 30]. At 6 months, the complete bone formation is characterized by extreme reduction or absence of the vascular signal as CPD could not penetrate the sound bone [27, 28]. After 1 week, a slight increase of the postsurgically bony defect may also occur, and this is explained by the curettage performed on the bony walls during surgical procedure [28]. It has been also noticed that early mineralization and bone deposition may be seen more precociously in USE than in CBCT [30] and traditional radiology [28]. As far as healing time, newly formed, scattered calcifications may be detected as early as 1-week postsurgery within the anechoic defects and can be interpreted as nucleation centers starting at the deepest point of the bony crypts [29].

Similarly to the surgical cases [27–29] and independently from the preoperative diagnosis, the lesions undergoing long-term healing following clinical treatment (RCT) at the B-mode USE exhibit the progressive decrease in size and the gradual increase in the echogenicity of the area, depicting the gradual maturation and mineralization of the bone matrix [31]. These changes in the grayscale signal support the evidence of the repair process and represent a common feature of hard tissue healing in orthopedics [38]. Conversely, the unhealed cases [24] show both the lack of transition in echogenicity and the increase in the size of the lesions. On the other hand, when a large periapical bone defect undergoing healing at 30–36 months was still showing hypoechoic areas within the new bone formation, the presence of apical scar tissue was suggested [25]. These last findings support the sensitivity of the USE to interpret the different responses of the bone, as demonstrated in previous studies on surgery in the jaws, where stagnation of fluids within the wound, lack of bone bridging, and absence of ossification were detected with ultrasounds [34, 35].

The CPD analysis of the vascular signal in the follow-up of the nonsurgical cases [24, 26, 27] highlighted a correlation between the decrease or loss of the vascular signal and the healing of the periapical lesions [24, 26]. In healed cases, the CPD signal may completely disappear within 6 months [27]. These data are in accordance with previous studies that demonstrated that the formation of a new cortical layer prevents ultrasound waves from reaching deeper tissues [38] and that during the bone remodeling process in fracture healing, no Doppler signal is present when a strong callus has developed [39]. Nevertheless, the continuous presence of vascularity at the CPD, after 6 months, within the context of a healing trend, is indicative of the lack of complete ossification [27]. It appears that the healing of apical granuloma is characterized by the progressive decrease of the vascular signal, at CPD, interpreted as the transition from an inflamed reactive tissue to a healthy connective tissue. These changes may be detected as early as 1 to 4 weeks [26] and at 3 months posttreatment [24]. The repair trend of the cystic lesions does not involve early modification of the vascular features at 1 to 4 weeks [24]. Instead, it is related to the proliferation of blood vessels within the former cystic areas at 6 weeks, followed by the progressive decrease of the CPD signal at 3 months, with the deposition of new bone material, until its disappearance at 6 months with the completion of the ossification.

To our knowledge, we are the first to perform a systematic review that assessed the effectiveness of USE to evaluate the healing of intra-osseous lesions of the jaws after both surgical and nonsurgical treatment providing the most comprehensive and highest level of evidence on this topic. The strength of this systematic review relies on its adherence to the PRISMA guidelines and to the a priori registration of the review protocol with the PROSPERO database, which seems to be associated with a higher quality reporting rate and a lower outcome reporting bias [40, 41].

Limitations

The main limitation of this systematic review comprised the heterogenicity among the studies performing USE to monitor the healing of intra-osseous bone lesions. Indeed, the individual study designs showed differences in factors concerning the sample characteristics such as patient features (sex, age) and the anatomic location of the lesions. Although a preoperative USE was performed at the beginning of each protocol, only 4 out of 7 studies attempted a preoperative diagnosis. As for the intra-operative factors, several differences in terms of the ultrasound devices used in the echographic examinations, as well as the type and position of the probe and frequency used, were present. Another main limitation was characterized by the lack of standardization in the follow-up time with the USE with protocols varying from 4 weeks to 36 months. New randomized clinical trials should be designed, taking into account the differences among the operators when performing further studies on this topic (radiologist vs dentist).

Conclusions

The USE implemented with CPD is an advanced imaging technique feasible to monitor the early and long-term response of the intra-osseous jaw lesions in both surgical and nonsurgical treatment. Although the heterogenicity in the study designs, the overall quality of the literature screened was high to moderate.

Clinical relevance

From the perspective of the growing attention towards minimally invasive dentistry and radiology, the possibility to introduce safe alternative imaging techniques not exposing the patient to ionizing radiations is of primary interest in the clinical practice. The results of our work demonstrate the effectiveness of USE in the follow-up of central lesions of both inflammatory and dysembryogenetic origin after treatment. In the near future, clinicians could experiment the introduction of the clinical echography in their daily practice to diagnose and monitor the intra-osseous bone lesions of the maxillary bones.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Davide Musu, Michela Boccuzzi, and Claudia Dettori. The first draft of the manuscript was written by Davide Musu and Michela Boccuzzi and subsequently revised by Elisabetta Cotti and Hagay Shemesh. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict(s) of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

References

1. MacDonald D (2016) Lesions of the jaws presenting as radiolucencies on cone-beam CT. Clin Radiol 71:972–985. https://doi.org/10.1016/j.crad.2016.05.018
2. Kanagasingam S, Lim CX, Yong CP, Mannocci F, Patel S (2017) Diagnostic accuracy of periapical radiography and cone beam computed tomography in detecting apical periodontitis using histopathological findings as a reference standard. Int Endod J 50:417–426. https://doi.org/10.1111/iej.12650
3. Torabinejad M, Rice DD, Maktabi O, Oyoyo U, Abramovitch K (2018) Prevalence and size of periapical radiolucencies using cone-beam computed tomography in teeth without apparent intraoral radiographic lesions: a new periapical index with a clinical recommendation. J Endod 44:389–394. https://doi.org/10.1016/j.joen.2017.11.015
4. European Society of Endodontology (2006) Quality guidelines for endodontic treatment: consensus report of the European Society of Endodontology. Int Endod J 39:921–930. https://doi.org/10.1111/j.1365-2591.2006.01180.x
5. AAE & AAOMR (2015) American Association of Endodontists and American Academy of Oral & Maxillofacial Radiology joint position statement: use of cone beam computed tomography in endodontics update. J Endod 41:1393–1396. https://doi.org/10.1016/j.joen.2015.07.013
6. Bender IB, Seltzer S (1961) Roentgenographic and direct observation of experimental lesions in bone. J Am Dent Assoc 87:708–716. https://doi.org/10.1097/00004770-200311000-00005
7. Bender IB, Seltzer S (1961b) The oral fistula: its diagnosis and treatment. Oral Surg Oral Med Oral Pathol 64:139–148. https://doi.org/10.1016/0030-4220(61)90270-5
8. Huumonen S, Ørstavik D (2002) Radiological aspects of apical periodontitis. Endod Top 1:3–25. https://doi.org/10.1034/j.1601-1546.2002.10102.x
9. Nair PN (1997) Apical periodontitis: a dynamic encounter between root canal infection and host response. Periodontol 2000(13):121–148. https://doi.org/10.1111/j.1600-0757.1997.tb00098.x
10. Abbott PV (2002) The periapical space–a dynamic interface. Aust Endod J 28:96–107. https://doi.org/10.1111/j.1747-4477.2002.tb00399.x
11. Nair PN, Sjögren U, Figdor D, Sundqvist G (1999) Persistent periapical radiolucencies of root-filled human teeth, failed endodontic treatments, and periapical scars. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 87:617–627. https://doi.org/10.1016/s1079-2104(99)70145-9

12. Nair PN (2006) On the causes of persistent apical periodontitis: a review. Int Endod J 39:249–281. https://doi.org/10.1111/j.1365-2591.2006.01099.x
13. Martin K (2011) Basic equipment, components and image production. In: Allan PL, Baxter GM, Weston MJ (eds) Clinical ultrasound, 3rd edn. Churchill Livingstone, Edinburg, pp 16–30
14. Cotti E, Campisi G, Garau V, Puddu G (2002) A new technique for the study of periapical bone lesion: ultrasound real time imaging. Int Endod J 35:148–152. https://doi.org/10.1046/j.1365-2591.2002.00458.x
15. Cotti E, Campisi G, Ambu R, Dettori C (2003) Ultrasound real-time imaging in the differential diagnosis of periapical lesions. Int Endod J 36:556–563. https://doi.org/10.1046/j.1365-2591.2003.00690.x
16. Cotti E, Simbola V, Dettori C, Campisi G (2006) Echographic evaluation of bone lesions of endodontic origin: report of two cases in the same patient. J Endod 32:901–905. https://doi.org/10.1016/j.joen.2006.01.013
17. Musu D, Rossi-Fedele G, Campisi G, Cotti E (2016) Ultrasonography in the diagnosis of bone lesions of the jaws: a systematic review. Oral Surg Oral Med Oral Pathol Oral Radiol 122:e19–e29. https://doi.org/10.1016/j.oooo.2016.03.022
18. Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 151:264–269. https://doi.org/10.7326/0003-4819-151-4-200908180-00135
19. Higgins JPT, Altman GD, Sterne ACJ Cochrane handbook for Systematic Review of Intervention Version 5.1.0 [updated March 2011]. The Chocrane Collaboration, 2011. Available from www.handbook.cochrane.org
20. Mallen C, Peat G, Croft P (2006) Quality assessment of observational studies is not commonplace in systematic reviews. J Clin Epidemiol 59:765–769. https://doi.org/10.1016/j.clinepi.2005.12.010
21. Downs SH, Black N (1998) The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health 52:377–384. https://doi.org/10.1136/jech.52.6.377
22. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses Available at http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
23. Maity I, Meena N, Kumari A (2014) Single visit non surgical endodontic therapy for periapical cyst: a clinical study. Contemp Clin Dent 5:195–202. https://doi.org/10.4103/0976-237X.132321
24. Maity I, Kumari A, Shukla AK, Usha HL, Naveen DN (2011) Monitoring of healing by ultrasound with color power Doppler after root canal treatment of maxillary anterior teeth with periapical lesions. J Conserv Dent 14:252–257. https://doi.org/10.4103/0972-0707.85804
25. Aggarwall V, Singla M (2010) Use of computed tomography scans and ultrasound in differential diagnosis and evaluation of nonsurgical management of periapical lesions. Oral Med Oral Pathol Oral Radiol Endod 109:917–923. https://doi.org/10.1016/j.tripleo.2009.12.055
26. Cotti E, Esposito SA, Musu D, Campisi G, Shemesh H (2018) Ultrasound examination with color power Doppler to assess the early response of apical periodontitis to the endodontic treatment. Clin Oral Investig 22:131–140. https://doi.org/10.1007/s00784-017-2089-z
27. Rajendran N, Sundaresan B (2007) Efficacy of ultrasound and color power Doppler as a monitoring tool in the healing of endodontic periapical lesions. J Endod 33:181–186. https://doi.org/10.1016/j.oen.2006.07.020
28. Tikku AP, Kumar S, Loomba K, Chandra A, Verma P, Aggarwal R (2010) Use of ultrasound, color Doppler imaging and radiography to monitor periapical healing after endodontic surgery. J Oral Sci 52:411–416. https://doi.org/10.2334/josnusd.52.411
29. Curvers F, Meschi N, Vanhoenacker A, Strijbos O, Van Mierlo M, Lambrechts P (2018) Ultrasound assessment of bone healing after root-end surgery: echoes back to patient's safety. J Endod 44:32–37. https://doi.org/10.1016/j.oen.2017.08.028
30. Zainedeen O, Haffar IA, Kochaji N, Wassouf G (2018) The efficacy of ultrasonography in monitoring the healing of jaw lesions. Imaging Sci Dent 48:153–160. https://doi.org/10.5624/isd.2018.48.3.153
31. Bruno C, Minniti S, Buttura-da-Prato E, Albanese M, Nocini PF, Pozzi-Mucelli R (2008) Gray-scale ultrasonography in the evaluation of bone callus in distraction osteogenesis of the mandible: initial findings. Eur Radiol 18:1012–1017. https://doi.org/10.1007/s00330-008-0856-6
32. Isaar I, Sahoo NK, Sinha R, Satija L, Chattopadhyay PK (2014) Comparative evaluation of the mandibular distraction zone using ultrasonography and conventional radiography. Int J Oral Maxillofac Surg 43:587–594. https://doi.org/10.1016/j.ijom.2013.11.016
33. Kaban LB, Thurmüller P, Troulis MJ, Glowacki J, Wahl D, Linke B, Rahn B, Perrott DH (2003) Correlation of biomechanical stiffness with plain radiographic and ultrasound data in an experimental mandibular distraction wound. Int J Oral Maxillofac Surg 32:296–304. https://doi.org/10.1054/ijom.2002.0380
34. O'Neill MJ (2005) Ultrasound evaluation of bone healing in distraction osteogenesis of the mandible. Atlas Oral Maxillofac Surg Clin North Am 13:63–67. https://doi.org/10.1016/j.cxom.2004.12.001
35. Thurmüller P, Troulis M, O'Neill MJ, Kaban LB (2002) Use of ultrasound to assess healing of a mandibular distraction wound. J Oral Maxillofac Surg 60:1038–1044. https://doi.org/10.1053/joms.2002.34417
36. Troulis MJ, Coppe C, O'Neill MJ, Kaban LB (2003) Ultrasound: assessment of the distraction osteogenesis wound in patients undergoing mandibular lengthening. J Oral Maxillofac Surg 61:1144–1149. https://doi.org/10.1016/s0278-2391(03)00672-4
37. Folestad A, Alund M, Asteberg S, Fowelin J, Aurell Y, Jan G, Cassuto J (2015) Offloading treatment is linked to activation of proinflammatory cytokines and start of bone repair and remodeling in Charcot arthropathy patients. J Foot Ankle Res 8:1–12. https://doi.org/10.1186/s13047-015-0129-y
38. Nocini PF, Albanese M, Wangerin K, Fior A, Trevisol L, Kretschmer W (2002) Distraction osteogenesis of the mandible: evaluation of callus distraction by ultrasonography. J Craniomaxillofac Surg 30:286–291. https://doi.org/10.1054/jcms.2002.0307
39. Risselada M, Kramer M, Saunders JH, Verleyen P, Van Bree H (2006) Power Doppler assessment of neovascularization during uncomplicated fracture healing of long bones in dogs and cats. Vet Radiol Ultrasound 47:301–306. https://doi.org/10.1111/j.1740-8261.2006.00144.x
40. Nagendrababu V, Pulikkotil SJ, Sultan OS, Jayaraman J, Peters OA (2018) Methodological and reporting quality of systematic reviews and meta-analyses in endodontics. J Endod 44:903–913. https://doi.org/10.1016/joen.2018.02.013
41. Sideri S, Papageorgiou SN, Eliades T (2018) Registration in the international prospective register of systematic reviews (PROSPERO) of systematic review protocols was associated with increased review quality. J Clin Epidemiol 100:103–110. https://doi.org/10.1016/jclinepi.2018.01.003

Chapter 8

General Discussion

This thesis aimed to evaluate the clinical applications of ultrasound examination to manage various intraosseous lesions affecting the jawbones. Ultrasound imaging provided reliable information on the content of lesions within the mandible and maxillary bones, with histopathological examination as a gold standard. Furthermore, the diagnostic ability of ultrasound showed its importance when the clinician was struggling to differentially diagnose lesions. The reliability of real-time ultrasound examination was confirmed in the management of complications of infective jaw lesions, since the technique could detect and trace the route of pus drainage (e.g., sinus tracts) from periapical lesions. The present work also demonstrated that, despite the limitations addressed in previous scientific literature, intra-osseous lesions in the jaws can be visualized using ultrasound in a predictable manner, independent of the diameter, thickness, and presence/absence of perforation in the cortical plate. In addition, the findings of the present thesis suggest that echography can be used to predict the outcome of both surgical and non-surgical treatment of bone lesions, as well as to monitor the long-term healing of such lesions.

Clinicians must accurately assess osteolytic lesions arising in the maxillary bones, because many such lesions are manifestations of benign or malignant neoplasms, although most have an inflammatory cause, especially those involving the periapex (Becconsall-Ryan *et al.* 2010, Huang *et al.* 2017). Furthermore, most non-endodontic lesions observed clinically and radiographically as sequelae of pulp necrosis can only be diagnosed retrospectively once a histological diagnosis has been made (Kontogiannis *et al.* 2015). **Chapters 2 and 3** highlighted how traditional radiological imaging and CT provide limited information on the content of lesions, especially if no contrast medium is used. In contrast, ultrasound examination provides information about the characteristics and vascularity of the pathological tissue within bone lesions. These technical features may play a major role in preliminary differential diagnosis of before histopathology is carried out, particularly since such lesions are often not easily or readily accessible to direct clinical examination. The evidence provided in **Chapters 2 and 3** was directly assessed by our research and clinical team, while in **Chapter 4**, we expanded the research to include the experience of other researchers by collecting all relevant clinical studies that focused on whether ultrasonography can be used in the differential diagnosis of jaw lesions—from case reports to non-randomized clinical studies. In all studies, histopathology evaluation was used as the gold standard reference.

The potential use of ultrasound imaging as a diagnostic aid in the maxillofacial area was further investigated in **Chapter 5**. Indeed, cutaneous sinus tracts of endodontic origin are often misdiagnosed and subjected to invasive and unnecessary interventions, such as surgical excisions and biopsies (Shobatake *et al.* 2014). The traditional diagnostic approach to disclose the origin and pathway of sinus tracts involves inserting an orthodontic wire or gutta-percha cone through the opening of the tract, followed by a periapical radiograph of the area (Bender & Seltzer 1961, Baumgartner *et al.* 1984). This procedure is invasive and can damage tissues, creating discomfort in the patient and stress in the clinician. For these reasons, other diagnostic tools, such as CBCT, have been investigated. Unfortunately, although CBCT is less invasive than the traditional procedure, it involves ionizing radiation and does not produce a direct image of the sinus tract. Instead, it only shows the pattern of the cortical plate perforation that leads to the periapical lesion (Shemesh *et al.* 2019). **Chapter 5** was the first experimental case-control study to show that ultrasound examination can directly visualize sinus tracts of endodontic origin and trace their pathways.

While **Chapters 2, 3**, **and 5** focused on the role of echographic imaging in the diagnosis of jawbone lesions, **Chapters 4 and 7** analyzed the prospective use of ultrasound examination to assess outcomes following treatment of these lesions. The rationale was that the longitudinal assessment of healing is traditionally based on clinical examination alongside follow-up radiographs (Zainedeen *et al.* 2018, Torabinejad *et al.* 2018). However, healing assessment using intraoral radiographs is based on the reduction of the radiolucent area, which represent the level of bone architecture destruction and mineral loss; little information can be obtained about dynamic host responses, such as vascular and cellular behavior in the affected area following treatment. This is important when no radiological evidence of complete bone healing is obtained, even in cases where remission of signs and symptoms has occurred, because no standardized radiological criteria are available to differentiate scar tissue from residual diseased tissue (Zainedeen *et al.* 2018, Marton & Kiss 2014). In addition, little information is available about the early response of periapical lesions to endodontic interventions; thus, the purpose of the clinical experiment in **Chapter 4** was to evaluate the effectiveness of color and power Doppler ultrasound to detect early changes in the size, echogenicity, and vascular supply of periapical tissues, as well as to predict the outcome of endodontic treatment. The rationale was that initial variations within and/or around the affected area may indicate the response of periapical tissues to a healing trend. **Chapter 7** was the second chapter of the present thesis to investigate the use of ultrasound imaging for follow-up of intraosseous lesions of the maxillary bones. As in **Chapter 3,** we expanded our research through systematic review, drawing on the best and most comprehensive available scientific evidence to ascertain whether ultrasound examination could depict both the early and the long-term responses to treatment of osteolytic lesions in the jaws, with traditional radiological and clinical examinations used as the gold standard. Thus, **Chapters 3 and 7** explored whether ultrasound examination could be used to diagnose and follow up intraosseous bone lesions in the jaws. These chapters highlighted both the strengths and the limitations of the technique, as addressed by different clinical studies in which the lesion could not be visualized. Several of these investigations objected to the use of ultrasound. However, the objections were mostly speculative, since they were not verified directly. **Chapter 6** clarified the limitations of ultrasound imaging. It was the first study to test the technique using an anatomical model that could simulate the physical obstacles that hinder clinicians during actual examination.

Different methodological approaches were chosen to provide a more extensive insight into the possible clinical applications of ultrasound examination for jawbone lesions.

Since **Chapters 3 and 7** summarized the two most relevant clinical applications of the technique, they were structured as systematic reviews of the literature. This allowed appropriate, evidence-based conclusions and recommendations to be drawn. To ensure that the findings of the systematic reviews were properly interpreted and applied to clinical practice, both protocols followed the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (Nagendrababu *et al.* 2018, Moher *et al.* 2009). The investigations of the literature were guided by the "PICO" questions, where the effectiveness of the ecographical interventions (I) was compared to the appropriate gold standards (C)—histopathology for differential diagnosis and long-term clinical and radiological follow-up for healing assessment. The studies had comparable research strategies within the electronic databases and collateral sources, as well as similar inclusion and exclusion criteria, data extraction, and data synthesis strategies. Specifically, only clinical studies on humans were included, and most were non-randomized clinical studies. In **Chapter 3**, eleven case reports were included, along with non-randomized clinical studies that had qualitative synthesis, to collect the most comprehensive available evidence. Case reports are generally considered low in the hierarchy of evidence, and they have low specificity for decision-making in healthcare. However, they can provide important or additional insights into everyday clinical practice and convey early information about unique or more effective management regimens, leading to improved decision-making about treatment options (Nagendrababu *et al.* 2018). In the present study, the quality of the case reports was critically appraised using the CAse REport (CARE) guidelines, which were published to standardize the reporting of case reports in medicine and surgery and ensure they were accurate, complete, and transparent (Gagnier *et al.* 2013). According to these principles, also **Chapter 2** was structured following the 13 items of the CARE checklist. The appraisal of the non-randomized clinical studies differed between the two chapters. In **Chapter 3**, the critical appraisal was performed to assess the directness of evidence and the risk of bias. In **Chapter 7**, the quality assessment was performed more extensively. The Newcastle–Ottawa Scale and the Downs and Black tool were used in conjunction, as suggested in the Cochrane Handbook, because no consensus statement is available about the appraisal of non-randomized clinical studies (Higgins *et al.* 2011). In addition, the Cochrane Collaboration's tool was used in both studies to assess the risk of bias. Overall, the studies reviewed in both chapters demonstrated a high to moderate quality and a low risk of bias. The results of the two chapters showed a positive correlation between the intervention investigated (ultrasound examination) and the outcome measures. Interestingly, in both protocols, the studies that focused on AP had comparable study designs and similar approaches to collecting and presenting the raw data, whereas the evidence available about other lesions of the jaws was not as robust, perhaps because AP is the most prevalent osteolytic lesion of the jawbones (Becconsall-Ryan *et al.* 2010, Bilodeau &Collins 2017). In contrast, the data collected in both chapters varied in terms of exposure and outcomes, so they could not be conflated into a meta-analysis; thus, they were summarized in a qualitative synthesis. Although the results of **Chapters 3 and 7** are promising, some articles may have been missed in the systematic search, especially articles among the gray literature or findings from non-English journals. Furthermore, studies involving a new diagnostic technique can be easily affected by publication bias, since studies presenting positive results are more likely to be published than those exhibiting null results (Dickersin 1990).

Three other chapters of the thesis were clinical studies performed on human patients, while **Chapter 6** was an *ex vivo* study. Ultrasound examination and analysis were always performed by an expert sonologist in **Chapters 2 and 4**. However, since the present work aimed to evaluate the possible introduction of ultrasound imaging in daily dental practice, the principal investigator in **Chapters 5 and 6** was one of the experimenters, under guidance from an experienced sonologist. Three distinct ultrasound machines were used for the different experiments. In **Chapters 4 and 5,** all echographic examinations were performed using either an Elegra

(Siemens, Erlangen, Germany) or an Aplio XG (Toshiba Medical Systems, Crawley, UK), which have regular or small, linear, high-definition, multifrequency ultrasound probes. As specified in the chapters, to ensure that both units were available during the main experiment, and to avoid confounding data, the two linear multifrequency probes of 7–9 MHz and 8–12 MHz were coupled with the Elegra Siemens and Toshiba Aplio XG. In this way, equivalent output scans could be generated during the calibration. In **Chapter 2**, only one lesion was assessed, and only the Toshiba Aplio XG apparatus was used with the ultrasound probe at 8–12 MHz. The choice of linear multifrequency probes in these experiments was justified, because lower frequencies can penetrate deeper within bone defects, whereas higher frequencies provide better resolution scans of early vascular and grayscale changes; they also permit soft tissues and sinus tracts to be visualized. In general, it is not the apparatus itself, but rather the setting of the probe with the machine that decides the quality and characteristics of the images. For these reasons, probes with specific characteristics were selected, after calibration within the two machines to obtain the same kind of images. Although several studies have investigated ultrasound imaging in endodontics and oral surgery (**Chapters 3 and 7**), there is currently no fixed standardization for the setting of probes, because a given frequency may be suitable with a machine but not another. A third device was used in **Chapter 6**: a Toshiba Medical-Aplio i700 (Toshiba, Tokyo, Japan), which has a high-frequency linear transducer at 4.5 MHz to increase the penetration of the ultrasonic waves through the cortical bone plates. In this chapter, the frequency of the probe was kept low because it was not necessary to obtain detailed images of changes within the lesions or soft tissue.

The scans collected from the real-time examinations in **Chapters 4–6** were submitted to a group of observers under the same conditions: TIFF images in a randomized order, organized as a PowerPoint presentation (Microsoft Office 2013; Microsoft Corporation, Redmond, WA, US) on a laptop computer (Dell Inc., Round Rock, TX).

In **Chapters 5 and 6**, the reliability of the readings was assessed through blinding of the observers, who were calibrated using images of subject material not included in the studies. In both experiments, the observers were calibrated using kappa statistics until they all obtained at least good agreement with the main examiner. In the present thesis, Cohen kappa values of 0.01–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, 0.81–0.99, and 1, represented slight, fair, moderate, substantial, almost perfect, and perfect agreement, respectively. In both studies, the blinded observers were asked to identify the presence or absence of a sinus tract or bone lesion, as well as to validate the diagnosis by tracing the boundaries of the lesions and sinus tracts on the echographic scans using a computer program. During the first session, the kappa value was used to determine how well the observers agreed with each other. To evaluate the inter-observer agreement a second time, as well as to establish intra-observer agreement, the same examination was repeated for 100% of the echographic scans after 2 weeks in **Chapter 5** and after 4 weeks in **Chapter 6**. The second session was performed by changing the randomized order of the scans and waiting a sufficient amount of time to limit recall bias. In both experiments, the inter-observer agreement was very high (from 0.86 and 0.89 in **Chapter 6** to 0.9 in **Chapter 5).** The intra-observer agreement was as high as 0.92 in **Chapter 6** and perfect (1.0) in **Chapter 5**. These slight differences between the two studies probably arose because a larger number of observations were made in

Chapter 6 than in **Chapter 5** (60 and 20, respectively), and because there was a greater number of blinded observers (3 *vs.* 2). These results are considerably better than those of conventional radiographs, where the 2D reading of a volume produces a discrepancy when considering inter-observer agreement, in which there is less than half agreement, and intra-observer agreement (72%–88%) in the diagnosis of an osteolytic area (Goldman *et al.* 1972, Goldman *et al.* 1974). Indeed, our agreement data are even higher than those of Tewary *et al.* (2011), who reported only a fair agreement (0.2–0.4) in the interpretation of digital radiographs using inter-observer examinations, with less than 25% agreement for all radiographs. In addition, the intra-observer reliability ranged from 41%–85%, with an average of 68% for all examiners (Tewary *et al.* 2011). In the present study, the inter- and intra-examiner agreement in **Chapters 5 and 6** were comparable to those reported by two *ex vivo* human studies that used CBCT (Patel *et al.* 2009, Al-Nuami *et al.* 2016), probably because both these studies were 3D in nature, as was the present study. Indeed, ultrasound examination is also called echo-tomography; thus, the image of the anatomy crossed by the ultrasound waves appears simple and easier to interpret than other 2D imaging methods.

In clinical studies, the examiners were able to detect all the lesions in real-time. However, there were some limitations in these results because both performance and detection bias were present in **Chapters 4 and 5**, the examiners were aware of the presence or absence of the lesion to be detected. In **Chapter 6**, the presence or absence of a lesion was not explicitly communicated to the examiners; however, these features were easily inferred from the characteristics of the anatomical blocks. Therefore, to evaluate the diagnostic accuracy of this imaging technique, statistical analysis was performed on the observations of the blinded observers. Our results showed that ultrasound examination was 100% accurate in diagnosing the absence of disease (100% specificity) and that it had an extremely low chance (0%; positive predictive value = of 1.0) of over-diagnosing the presence of disease. In addition, the accuracy of the two studies was comparable, with mean values of 97.5 in **Chapter 5** and 97.8 in **Chapter 6**, perhaps because the observers underwent calibration training and exercises prior to the ultrasonographic assessment, which consolidated their ability to identify both the sinus tracts and the bone defects. In **Chapter 6**, in the presence of sound bone intra-bony lesions, complete reflection of the ultrasound waves occurred. Nevertheless, ultrasound examination revealed lower values of sensitivity (95%–97.3%) and negative predictive value (0.89–0.96), demonstrating that it can accurately diagnose the incidence of lesions, but that false negatives conferred a risk of missing disease in 4%–11% of cases. Despite these shortcomings, the accuracy, sensitivity, and negative predictive value were higher than those reported for digital and film periapical radiographs, and similar to those reported for CBCT (Leonardi Dutra *et al.* 2016, Kanagasingam *et al.* 2017a, Stavropoulos &Wenzel 2007). In addition, negative controls were used in all the experimental protocols of **Chapters 4–6**, since a diagnostic test should detect disease where it is present and exclude healthy subjects, thus establishing an appropriate reference standard (Pope *et al.* 2014, Khanaghasingam et al 2017b).

The low number of cases was the main limitation to the statistical significance of the studies in **Chapters 4 and 5**. Nonetheless, to assess the power of the associations obtained through ultrasound examination, a statistical test was performed; given the small number of observations, the Fisher's exact test was chosen.

Indeed, this test requires 2 x 2 contingency tables, a number of observations < 30, and cells with expected frequencies < 5. The P-value was set to < 0.05, since statistical convention states that studies with a small sample size have a 5% probability of refusing the null hypothesis. In **Chapter 6**, the significance of the findings was appraised using chi-square statistics with the Yates correction. This correction was applied considering the total number of observations (n = 60). Even in this chapter, a P-value < 0.05 was considered statistically significant.

Conclusions and future research

In recent years, clinicians have shown increasing interest in minimally invasive dentistry and radiology, and many have sought safe, alternative imaging techniques that do not expose the patient to ionizing radiation. The results of this thesis validated the effectiveness of ultrasound examination and, in the near future, clinicians could introduce clinical echography into their daily practice to diagnose and monitor intraosseous maxillary and mandibular lesions. If perfected, the technique could also be used in experimental studies to evaluate the response of jawbone lesions to different treatment products and techniques. However, further investigations are needed to standardize the procedure before it can be introduced into conventional dental examination.

References

Al-Nuaimi N, Patel S, Foschi F, Mannocci F (2016) The detection of simulated periapical lesions in human dry mandibles with cone beam computed tomography – a dose reduction study. *International Endodontic Journal* **49**, 1095–104.

Baumgartner JC, Picket AB, Muller JT (1984) Microscopic examination of oral sinus tracts and their associated periapical lesions. *Journal of Endodontics* **10**, 146–52.

Becconsall-Ryan K, Tong D, Love RM (2010) Radiolucent inflammatory jaw lesions: a twenty-year analysis. *International Endodontic Journal* **43**, 859-65.

Bender IB, Seltzer S (1961) The oral fistula: its diagnosis and treatment. *Oral Surgery Oral Medicine Oral Pathology* **64**, 139–48.

Bilodeau EA, Collins BM (2017) Odontogenic Cysts and Neoplasms. *Surgical Pathology Clinics* **10**, 177-222.

Dickersin K (1990) The existence of publication bias and risk factors for its occurrence. *Journal of the American Medical Association* **263**, 1385-9.

Gagnier JJ, Kienle G, Altman DG, Moher D, Sox H, Riley D, CARE Group (2013) The CARE guidelines: consensus-based clinical case reporting guideline development. *Headache* **53**, 1541-7.

Goldman M, Pearson AH, Darzenta N (1972) Endodontic success – who's reading the radiograph? *Oral Surgery Oral Medicine Oral Pathology* **33**, 432–7.

Goldman M, Pearson AH, Darzenta N (1974) Reliability of radiographic interpretations. *Oral Surgery Oral Medicine Oral Pathology* **38**, 287–93.

Huang HY, Chen YK, Ko EC, Chuang FH, Chen PH, Chen CY, Wang WC (2017) Retrospective analysis of nonendodontic periapical lesions misdiagnosed as endodontic apical periodontitis lesions in a population of Taiwanese patients. *Clinical Oral Investigation* **21**, 2077-82.

Higgins JPT, Altman GD, Sterne ACJ (2011) Cochrane handbook for Systematic Review of Intervention Version 5.1.0 [updated March 2011]. The Chocrane Collaboration.

Kanagasingam S, Hussaini HM, Soo I, Baharin S, Ashar A, Patel S (2017a) Accuracy of single and parallax film and digital periapical radiographs in diagnosing apical periodontitis - a cadaver study. *International Endodontic Journal* **50**, 427-36.

Kanagasingam S, Lim CX, Yong CP, Mannocci F, Patel S (2017b). Diagnostic accuracy of periapical radiography and cone beam computed tomography in detecting apical periodontitis using histopathological findings as a reference standard. *International Endodontic Journal* **50**, 417-26.

Kontogiannis TG, Tosios KI, Kerezoudis NP, Krithinakis S, Christopoulos P, Sklavounou A (2015) Periapical lesions are not always a sequelae of pulpal necrosis: a retrospective study of 1521 biopsies. *International Endodontic Journal* **48**, 68-73.

Marton IJ, Kiss C (2014) Overlapping protective and destructive regulatory pathaways in apical periodontitis. *Journal of Endodontics* **40**, 155–63.

Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medicine* **151**, 264-9.

Nagendrababu V, Pulikkotil SJ, Sultan OS, Jayaraman J, Peters OA (2018) Methodological and Reporting Quality of Systematic Reviews and Meta-analyses in Endodontics. *Journal of Endodontics* **44**, 903-13.

Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH (2019) Guidelines for reporting the quality of clinical case reports in Endodontics: a development protocol. *International Endodontic Journal* **52**, 775-8.

Nardi C, Calistri L, Pradella S, Desideri I, Lorini C, Colagrande S (2017) Accuracy of orthopantomography for apical periodontitis without endodontic treatment. *Journal of Endodontics* **43**, 1640-6.

Patel S, Dawood A, Mannocci F, Wilson R, Pitt Ford T (2009) Detection of periapical bone defects in human jaws using cone beam computed tomography and intraoral radiography. *International Endodontic Journal* **42**, 507-15.

Pope O, Sathorn C, Parashos P (2014) A comparative investigation of cone-beam computed tomography and periapical radiography in the diagnosis of a healthy periapex. *Journal of Endodontics* **40**, 360-5.

Shemesh A, Hadad A, Azizi H, Lvovsky A, Ben Itzhak J, Solomonov M (2019) Cone-beam computed tomography as a noninvasive assistance tool for oral cutaneous sinus tract diagnosis: a case series. *Journal of Endodontic*s **45**, 950-6.

Shobatake Chinatsu, Miyagawa Fumi, Fukumoto Takaya, Hirai Toshiko, Kobayashi Nobuhiko, Asada Hideo (2014) Usefulness of ultrasonography for rapidly diagnosing cutaneous sinus tracts of dental origin. *European Journal of Dermatology* **24**, 683–7.

Stavropoulos A, Wenzel A (2007) Accuracy of cone beam dental CT, intraoral digital and conventional film radiography for the detection of periapical lesions: an ex vivo study in pig jaws. *Clinical Oral Investigations* **11**, 101–6.

Tewary S, Luzzo J, Hartwell G (2011) Endodontic radiography: who is reading the digital radiograph? *Journal of Endodontics* **37**, 919-21.

Torabinejad M, Rice DD ,Maktabi O, Oyoyo U, Abramovitch K (2018) Prevalence and size of periapical radiolucencies using cone-beam computed tomography in teeth without apparent intraoral radiographic lesions: a new periapical index with a clinical recommendation. *Journal of Endodontics* **44**, 389-94.

Zainedeen O, Haffar IA, Kochaji N, Wassouf G (2018) The efficacy of ultrasonography in monitoring the healing of jaw lesions. Imaging Science in Dentistry **48**, 153-60.

Summary and conclusions

Samenvatting en conclusies

Summary and conclusions

This thesis focused on the clinical applications of ultrasound examination to manage intraosseous jawbone lesions. The various protocols of this work were conducted with several different study designs to confer a better understanding of the potential of this imaging technique, as well as a robust insight into its strengths and limitations.

Chapter 2 followed the CARE guidelines to report the management of a complex case of traumatic bone cyst combined with apical periodontitis, wherein a multi-modular diagnostic assessment was performed, which included ultrasound examination. The study found that, through real-time ultrasound examination with color and power Doppler, the lesion was visualized as cystic (transonic/anechoic, well-defined cavity), with no evidence of central vascularization and a strong peripheral power Doppler signal indicating a layer of vascularized and inflamed tissue in the walls of the lesion. All these features were confirmed following surgical exploration and biopsy. The study demonstrated a positive correlation between echographic diagnosis and histopathological findings.

Chapter 3 carried out a systematic review that aimed to analyze the efficacy of ultrasonography in the differential diagnosis of jaw lesions, using histopathology evaluation as the gold standard reference. The study sought to define the potential of this tool in the oral and maxillofacial regions. A total of 704 records were identified through electronic searches (503 from PubMed's MEDLINE, 59 from Scopus, 116 from Embase, and 26 from BIOSIS citation index). Once duplicates were removed, the titles and abstracts were screened. Next, 64 full-text papers were assessed for eligibility, and 22 full-text articles were included in the qualitative synthesis, according to the inclusion and exclusion criteria. The study determined that ultrasound examination has been used effectively to diagnose infective and/or inflammatory lesions, cysts, non-odontogenic tumors, odontogenic tumors, and arteriovenous malformations, as well as to differentially diagnose lesions of endodontic origin, with histological analysis as the gold standard. These findings confirmed that ultrasound examination is a viable adjunct to other special tests for diagnosing intraosseous lesions of the jaws, as it is non-invasive and does not involve ionizing radiation exposure.

Chapter 4 aimed to assess the possibility of detecting early vascular changes in apical periodontitis using color-power Doppler ultrasound examination, as well as to establish a correlation between the early response of periapical lesions to treatment and subsequent healing. Periapical lesions were examined using echography before endodontic treatment, when a preoperative differential diagnosis between cystic and granulomatous lesions was performed. The examination was repeated 1 week after the first access to the endodontic system, as well as 4 weeks after the completion of the endodontic treatment. The vascular modifications were then correlated with long-term clinical and radiographic follow-up. It was concluded that the decrease or disappearance of vascular flow observed 4 weeks after endodontic treatment was significantly related to healing of the lesions. However, combining the data with the preoperative echographic diagnosis, this association was only demonstrated for apical granuloma.

The aim of **Chapter 5** was to evaluate whether sinus tracts of endodontic origin could be detected and their routes traced using real-time ultrasound examination. To our knowledge, this was the first experimental study to assess such an application of ultrasonography. In the experiment, the two calibrated examiners performed echography on 10 patients who had apical periodontitis with a sinus tract and 10 control patients who had a periapical disease without a sinus tract. The images were then submitted to two blinded observers, who were asked to diagnose the presence of a sinus tract and to trace it with a computer program. The study showed that the examiners detected apical periodontitis in all examinations, and that they traced all the pathways of the sinus tracts in real time. The technique showed very high intra- and inter-observer agreement between the blinded observers. Data analysis showed that ultrasound examination is an accurate technique to describe and trace sinus tracts of endodontic origin. In addition, the 3D mode enabled reconstructions of more complex paths, and the color-power Doppler ultrasound revealed the vascularity surrounding the lesions within the soft tissues.

Chapter 6 aimed to evaluate the accuracy of ultrasound examination to detect small and large artificial bone defects with incomplete erosion of the cortical bone plate, as well as to determine the minimum cortical thickness that constitutes a barrier for ultrasound waves. To our knowledge, this was the first *ex vivo* study evaluating this association. Sixty bovine anatomical blocks were harvested and uniformly distributed among the six experimental groups. Two examiners could detect all bone defects with ultrasound examination performed in real time. Three calibrated, blinded examiners analyzed the scans independently and demonstrated high reliability. The ultrasound examination obtained very high values of accuracy, sensitivity, and negative predictive value, as well as a perfect score for specificity and positive predictive value when diagnosing the presence or absence of artificial intraosseous lesions in the anatomical blocks. In addition, the experiment demonstrated that bone lesions in the jaws can be detected using echography regardless of their diameter, the presence or absence of cortical plate perforation, or its thickness.

Chapter 7 sought to assess the potential of ultrasound examination as an adjunctive method to conventional radiology to evaluate healing after the treatment of jawbone lesions. The study protocol was registered with PROSPERO and involved six electronic databases (PubMed, Web of Science, SCOPUS, Embase, Cochrane Library, and Best Evidence). A total of 4404 records were screened, and seven studies meeting the inclusion criteria were included in the systematic review. Ultrasound examination showed effectiveness in monitoring healing of lesions after non-surgical treatment in human patients. Specifically, it showed reductions in size, increases in echogenicity, and progressive decreases or disappearances of vascular flow within the lesions. Similar results were obtained when evaluating the affected area after surgical excision of the lesions. Indeed, echographic assessment using color and power Doppler showed an initial increase in the size and vascularity of the bone cavities, followed by a progressive reduction in size, a gradual increase in echogenicity (from anechoic to hyperechoic) and a progressive decrease of the vascular signal until it disappeared. This was the first systematic review to produce evidence that color-power Doppler echography can monitor the early and long-term response of intraosseous lesions in the jaws to both surgical and non-surgical treatments.

The present thesis collected the best available evidence concerning the use of ultrasonography to manage of jawbone lesions. New clinical research should be carried out into the use of ultrasonography among dental professionals on a daily basis, and researchers should seek to improve the availability of echographic machines and probes that are suitable for oral application.

Samenvatting en conclusies

Dit proefschrift richt zich op de klinische toepassingen van echografisch onderzoek bij kaakbotlaesies. De verschillende protocollen voor dit werk zijn doorgevoerd aan de hand van een aantal verschillende onderzoeksopzetten, om zo beter inzicht te krijgen in de mogelijkheden van deze beeldvormende techniek en om gedegen kennis te verwerven over de voordelen en beperkingen ervan.

In **hoofdstuk 2** was het doel om te beschrijven hoe een complexe casus op grond van de CARE-richtlijnen kan worden aangepakt. In de casus ging het om een traumatische botcyste in combinatie met apicale parodontitis, waarbij een multi-modulaire diagnostische beoordeling werd gemaakt, inclusief echografisch onderzoek. In dit onderzoek werd gemeld dat, door middel van real-time onderzoek met behulp van kleuren- en powerdopplerechografie, de laesie in beeld kon worden gebracht als cystisch (transsoon/anechoïsch duidelijk gedefinieerde caviteit) zonder aantoonbare centrale vascularisatie en met een sterk perifeer powerdopplersignaal. Op basis hiervan kon een gevasculariseerde en ontstoken weefsellaag worden vastgesteld in de wanden van de laesie. Al deze eigenschappen werden vervolgens door middel van chirurgisch onderzoek en biopsie bevestigd. Bij dit onderzoek werd er een positieve correlatie gevonden tussen de echografische diagnose en het histopathologische bevindingen.

In **hoofdstuk 3** werd er een systematische review uitgevoerd, met als doel om de vast te stellen hoe effectief echografie is bij de differentiële diagnose van kaaklaesies, aan de hand van histopathologisch onderzoek als gouden standaard ter referentie. Op die manier werd het gebruik van dit instrument voor het orale en maxillofaciale gebied beoordeeld. Door middel van elektronische zoekopdrachten werden er in totaal 704 publicaties opgespoord (503 op MEDLINE van PubMed, 59 op Scopus, 116 op Embase en 26 van de citeringsindex BIOSIS). Na het uitsorteren van dubbelingen werden de publicaties gescreend op titel en samenvatting. Van 64 volledige publicaties werd beoordeeld of ze in aanmerking kwamen, en vervolgens werden er op basis van inclusie- en exclusiecriteria 22 volledige artikelen geselecteerd voor de kwalitatieve synthese. Uit dit onderzoek kwam naar voren dat echografisch onderzoek effectief is toegepast voor de diagnose van infecties en/of ontstekingslaesies, cysten, niet-odontogene tumoren, odontogene tumoren en arterioveneuze misvormingen en ten behoeve van de differentiële diagnose van laesies van endodontische oorsprong, vergeleken met de gouden standaard van histologische analyse. Uit deze resultaten kwam dan ook naar voren dat echografisch onderzoek een zinnige toevoeging is aan het spectrum van tests voor de diagnose van intraossale kaaklaesies, aangezien het gaat om een non-invasieve techniek, waarbij de patiënt niet wordt blootgesteld aan ioniserende straling.

In **hoofdstuk 4** is beoordeeld of het mogelijk is om vroege vasculaire veranderingen bij apicale parodontitis vast te stellen met behulp van echografisch onderzoek met kleurenpowerdoppleronderzoek en om een correlatie vast te stellen tussen de vroege respons van de peri-apicale laesies op behandeling en de mogelijke genezing ervan. De peri-apicale laesies werden voorafgaand aan de endodontische behandeling zichtbaar gemaakt door middel van echografie. Het onderzoek werd 1 week na de eerste zitting van de endodontische behandeling herhaald en 4 weken na afronding van de endodontische behandeling nogmaals. De vasculaire aanpassingen werden vervolgens gecorreleerd aan klinische en radiologische langetermijn-follow-up. Er werd vastgesteld dat er bij de afname of het verdwijnen van vasculaire stroming in de 4 weken na de endodontische behandeling sprake was van een significant verband met de genezingstrend van de laesies. Bij het combineren van de gegevens met de pre-operatieve echografische diagnose was dit verband er alleen bij apicaal granuloom.

Het doel van **hoofdstuk 5** was het beoordelen van de mogelijkheid om fistels van endodontische oorsprong op te sporen en hun verloop te achterhalen met behulp van echografisch real-time-onderzoek. Dit is naar ons weten het eerste experimentele onderzoek waarbij een dergelijke toepassing van echografie is onderzocht. Bij het experiment voerden de beide gekalibreerde onderzoekers echografisch onderzoek uit bij tien patiënten met apicale parodontitis met een fistel en bij tien controlepatiënten met een peri-apicale aandoening zonder fistel. De afbeeldingen werden vervolgens geblindeerd naar twee observatoren gestuurd, met het verzoek tot het diagnosticeren van een fistel en het markeren daarvan in een computerprogramma. In dit onderzoek is te zien dat de onderzoekers bij alle onderzochte gevallen de apicale parodontitis vaststelden en het verloop van alle fistels in real-time markeerden. Bij de techniek was sprake van een zeer hoge overeenstemming bij de observatoren zelf en tussen de observatoren onderling ten aanzien van de geblindeerde afbeeldingen. Uit de analyse van de gegevens bleek dat echografisch onderzoek een nauwkeurige techniek is voor het omschrijven en opsporen van fistels van endodontische oorsprong. Daarnaast waren door toepassing van de 3-D-modus reconstructies van de complexere fistel routes mogelijk en maakte de toepassing van kleurenpowerdoppler de vascularisatie van de omringende laesies binnen de weke delen zichtbaar.

In **hoofdstuk 6** werd gekeken naar de nauwkeurigheid van echografisch onderzoek bij het detecteren van kleine en grote kunstmatige botdefecten bij afwezigheid van complete erosie van de corticale botplaat en het bepalen van de minimale dikte die het corticale bot moet hebben om een barrière voor geluidsgolven te vormen bij echografie. Tot nu toe was dit het eerste ex-vivo-onderzoek waarbij dit verband werd onderzocht. Voor dit experiment werden er zestig anatomische blokken van boviene oorsprong geoogst en evenredig verdeeld over zes onderzoeksgroepen. Twee onderzoekers konden alle botdefecten vaststellen door middel van real-time echografisch onderzoek. Drie gekalibreerde observatoren voerden onafhankelijk van elkaar een analyse uit van de geblindeerde scans, waarbij sprake was van een hoge betrouwbaarheid van de uitslagen die met de techniek werden bepaald. Het echografische onderzoek behaalde zeer hoge waarden ten aanzien van nauwkeurigheid, sensitiviteit en negatieve voorspellende waarde en een perfecte score voor specificiteit en positieve voorspellende waarde bij de diagnose van aan- of afwezigheid van kunstmatige intraossale laesies in de anatomische blokken. Daarnaast kon op basis van het huidige experiment worden aangetoond dat botlaesies in de kaken kunnen worden vastgesteld met behulp van echografie – ongeacht hun diameter en ongeacht de aan- of afwezigheid van perforaties in de corticale botplaat of de dikte van de plaat.

Hoofdstuk 7 had tot doel om te beoordelen welk potentieel echografisch onderzoek heeft als aanvullende methode naast conventionele radiologie, als het gaat om de beoordeling van genezingsprocessen die optreden na de behandeling van botlaesies van de kaken. Het onderzoeksprotocol is geregistreerd door middel van PROSPERO en er werden 6 elektronische databanken (PubMed, Web of Science, Scopus, Embase, Cochrane Library en Best Evidence) doorzocht. Er werden in totaal 4404 publicaties gescreend en bij de systematische beoordeling werden 7 onderzoeken geselecteerd die voldeden aan de inclusiecriteria. Het echografische onderzoek bleek een effectieve methode voor het observeren van de genezing van de laesies na niet-chirurgische behandeling van menselijke patiënten, in de vorm van een afname in grootte van het aangetaste gebied, een toename van de echogeniciteit en de progressieve afname of het verdwijnen van het vasculaire stromingssignaal binnen de laesies. Vergelijkbare resultaten werden behaald bij de beoordeling van het aangetaste gebied na chirurgische excisie van de laesies. Bij beoordeling van de resultaten van echografisch onderzoek was eerst sprake van een toename in grootte en vasculariteit van de botcaviteiten, daarna was er echter sprake van een progressieve afname in grootte, een geleidelijke toename van de echogeniciteit (van anechoïsch naar hyperechoïsch) en een progressieve afname van het vasculaire signaal op de kleuren- en powerdoppler, waarbij het signaal uiteindelijk helemaal verdween. Dit was de eerste systematische beoordeling die aantoonde dat de toepassing van echografie met behulp van kleurenpowerdoppler een geavanceerde beeldvormende techniek is voor het observeren van vroege respons en langetermijnrespons van intraossale kaakbotlaesies bij zowel chirurgische als niet-chirurgische behandelingen.

In dit proefschrift zijn de beste beschikbare bewijzen verzameld voor het gebruik van echografie bij kaakbotlaesies. Door middel van nieuw klinisch onderzoek kan het dagelijks gebruik ervan onder tandartsen en mondzorg professionals worden beoordeeld en kan de beschikbaarheid van echografische apparatuur en probes voor oraal gebruik verder worden verbeterd.

About the Author

Davide Musu was born on October 9, 1990, in Sardinia, Italy. He graduated from high school in 2009. He graduated with honors as a dentist from the Dental School of the University of Cagliari in 2016. During his undergraduate education started his activity of research obtaining a grant as a visiting researcher at the University of Adelaide, Australia in 2015. In 2017 he obtained his Master in "Clinical and Surgical Endodontics" at the University of Cagliari, Italy. In 2018 he started his research program as PhD candidate in the Department of Endodontology, ACTA, the University of Amsterdam (UvA), The Netherlands. Additionally, he has been working as a general dental practitioner in a private practice since 2017.

List of Publications

Musu D, Bardini G, Shemesh H, Dettori C, Cotti E. Multimodular Assessment of a Traumatic Bone Cyst Overlapped with Apical Periodontitis. Case Rep Dent. 2020 November.

Bardini G, Casula L, Ambu E, **Musu D,** Mercadè M, Cotti E. A 12-month follow-up of primary and secondary root canal treatment in teeth obturated with a hydraulic sealer. Clin Oral Investig. 2020 Sep 28.

Musu D, Cadeddu Dessalvi C, Shemesh H, Frenda MG, Mercuro G, Cotti E. Ultrasound examination for the detection of simulated periapical bone lesions in bovine mandibles: an ex vivo study. Int Endod J. 2020 Sep;53(9):1289-1298.

Musu D, Shemesh H, Boccuzzi M, Dettori C, Cotti E. The effectiveness of ultrasound examination to assess the healing process of bone lesions of the jaws: a systematic review. Clin Oral Investig. 2020 May 16.

Casula L, Cotti E, Capparè P, **Musu D.** Transient discoloration and apical breakdown after trauma in permanent dentition. J Biol Regul Homeost Agents. 2019 September-October,33(5):1647-1650.

Cotti E, **Musu D**, Goddi A, Dettori C, Campisi G, Shemesh H. Ultrasound Examination to Visualize and Trace Sinus Tracts of Endodontic Origin. J Endod. 2019 Oct;45(10):1184-1191.

Musu D, Cotti E. Traumatic bone cyst of the jaws: an overview. J Biol Regul Homeost Agents. 2019 Jul-Aug;33(4):1261-1263.

Peddis N, **Musu D,** Ideo F, Rossi-Fedele G, Cotti E. Interaction of biologic therapy with apical periodontitis and periodontitis: a systematic review. Aust Dent J. 2019 Jun;64(2):122-134.

Heithersay GS, **Musu D,** Cotti E. External tooth resorption associated with a peripheral odontogenic fibroma: review and case report. Aust Dent J. 2017 Dec;62(4):516-522.

Cotti E, Esposito SA, **Musu D,** Campisi G, Shemesh H. Ultrasound examination with color power Doppler to assess the early response of apical periodontitis to the endodontic treatment. Clin Oral Investig. 2018 Jan;22(1):131-140.

Peddis N, Ideo F, **Musu D**, Rossi-Fedele G, Cotti E. The interrelationships of biologic medications with periodontitis and apical periodontitis: a systematic review. J Endod (Supplement) 2017;43:e49.

Musu D, Rossi-Fedele G, Campisi G, Cotti E. Ultrasonography in the diagnosis of bone lesions of the jaws: a systematic review. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016 Jul;122(1):e19-29.

Rossi-Fedele G, **Musu D,** Cotti E, Doğramacı EJ. Root Canal Treatment versus Single-Tooth Implant: A Systematic Review of Internet Content. J Endod. 2016 Jun;42(6):846-53.

Other Publications

Musu D, Pinna C, Orani L, Ambu R. Reactive focal fibrous hyperplasia: clinical case and report of the literature. Dental Accademy 2014. Online Italian Journal.

Musu D, Caria C, Pinna C, Songini A, Spinas E. Review: traumatic avulsion in deciduous dentition. Dental Accademy 2014. Online Italian Journal.

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