EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

INSTITUTO UNIVERSITÁRIO EGAS MONIZ

DOUTORAMENTO EM CIÊNCIAS BIOMÉDICAS

SEX AND AGE PREDICTION THROUGH DENTAL AND RADIOGRAPHIC MEASUREMENTS: ELABORATION AND VALIDATION OF FORENSIC MODELS FOR THE PORTUGUESE POPULATION

Trabalho submetido por
João Albernaz Santos Coelho das Neves
para a obtenção do grau de Doutor em Ciências Biomédicas

Trabalho orientado por
Prof. Doutor José João Baltazar Mendes

janeiro de 2024

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e coorientado por
Prof. Doutor João Tiago da Silva Botelho

janeiro de 2024

DEDICATÓRIA

“Behind every young child who believes in himself are the parents who believed first.”

Mathew Jacobson

Aos meus Pais e à Tia Bel, que são os melhores exemplos que poderia imaginar ter. Todos os valores que me caracterizam foram-me transmitidos pelos meus Pais e Tia, com o seu carinho, dedicação, ética, responsabilidade e amor.

Espero alcançar metade do sucesso que eles alcançaram e assim terei a certeza de que fiz a diferença.

Ao meu Pai, Fernando, um verdadeiro exemplo de um bom ser humano, o meu herói e *role model*.

À minha Mãe, Isabel, pelo altruísmo e carinho que sempre demonstrou, permitindo-me crescer num ambiente seguro e familiar.

À minha Tia, Bel, por ser a minha segunda Mãe, que é a única forma que consigo descrever o que a minha tia significa para mim.

Tão importante como quem me criou é quem está ao meu lado no dia-a-dia.

À minha querida mulher, Maria, uma verdadeira Super-Mulher que foi o meu porto seguro, especialmente nestes 4 anos, uma constante apoiante e a voz da razão sempre que precisei.

Aos meus filhos, Manuel, Francisco e António, vocês são o futuro e o melhor de mim e da Mãe. Cresçam, sejam felizes e sigam as pisadas que os vossos avós e pais deram, tenham muito orgulho neles tal como nós temos em vocês.

ACKNOWLEDGEMENTS

To Prof. Dr. José João Mendes, for his mentorship, dedication and encouragement to make this project a reality. A true commander who knew how to encourage me at the right times and give me a break when the pressure was on. I have him to thank for all the opportunities I have had in my academic career, and I hope to live up to his expectations.

To Dr. João Rua, for his ability to share knowledge and opinions in his light-hearted and witty way, making Mondays more interesting.

To Prof. Dr. Ana Delgado, always very helpful and kind, never refusing to help when needed.

To Vanessa Machado and João Botelho, who went from students to colleagues, from acquaintances to great friends, from good advisors to personal references. Witnessing your growth has been a source of pride, and I hope to continue growing with you and your example.

To Luísa Bandeira Lopes, who was my teacher and later my friend. After a few collaborations, I always know that the next one will be even better.

To Dr. Mário Rito Pereira and Dr. Diogo Guerreiro, the first mentors in my clinical field, who gave me the passion for my work that I still feel to this day.

To Prof. Dr. Cristina Manso, for being part of my academic journey, always treating me with respect and kindness, and always taking care to make us feel valued.

To Prof. Dr. Nuno Taveira for the academic opportunity presented, always giving us the best feedback so that we could bring this project to a successful conclusion.

To Prof. Dr. Luís Proença, an invaluable and infinite help in a field as interesting as statistics. No project should be started without being in tune with the statistical part, and fortunately Prof. Dr. Proença has the talent of simplifying the process, asking the right questions, and making the result transparent for us, clinicians.

To the Department of Conservative Dentistry, under the supervision of Prof. Dr. Ana Mano Azul. Perhaps the first person to encourage me and give me the research "bug", more than 10 years ago. She showed me that it always takes perseverance, will and commitment to research projects to be proud of them later.

To the Endodontics team, professors, MDFVs and monitors for all their help over the years, making the evenings much easier and more enjoyable.

To my PhD colleagues of the last four years, João Gaspar, José Maria Cardoso and Pedro Rodrigues, for your companionship, friendship and shared "suffering". Each of your projects is excellent and you deserve every success.

To the AM Smiling team, led by Dr. Ana Mexia, a long relationship of respect, mutual growth and friendship. I owe much of who I am professionally to this fantastic team that has put up with me for over 10 years.

To Leonel Lima, who helped me a lot at the “finishing line”, making this word presentable and ready to submit.

To my friends who have put up with me so much over the last few years, Sofia, Rodrigo, Marta, Francisco, Ricardo and so many others who have made this endeavor much easier and more doable and helped me keep my "sanity".

Thank you all so much!

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Declaro, por minha honra, que o presente trabalho académico é original e foi elaborado por mim próprio (a), não se tendo recorrido a quaisquer outras fontes, para além das indicadas, usadas, adotadas literalmente ou adaptados a partir dos seus originais (em fontes impressas, não impressas ou na internet) e encontram-se adequados, identificados e citados, com observância das convenções do trabalho académico em vigor.

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Declaração de Financiamento

Código | IMP-EM-EI-14

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Data: 9/11/2023

RESUMO

O interesse pela estimativa da idade tem vindo a aumentar nos últimos anos, quer devido ao número crescente de cadáveres não identificados, quer devido à existência de pessoas vivas sem documentos validados. Existem dois tipos de estimativa de idade dentária: as alterações de desenvolvimento, que ocorrem na dentição humana durante a erupção, e as alterações degenerativas, que ocorrem com o desgaste na mastigação. A erupção do terceiro molar ocorre entre os 17 e 19 anos de idade. O dente pode estar completamente desenvolvido, mas mal posicionado, total ou parcialmente, ou mesmo ausente. Apenas as radiografias podem fornecer uma documentação exata do seu estado. Adicionalmente, a determinação do sexo é importante na medicina dentária forense para ajudar a identificar pessoas desconhecidas. Existem quatro formas de o fazer: análise do crânio, análise do ADN do tecido oral, morfologia dentária e medição das dimensões vestibulares mesiodistais e linguais, sendo esta última a forma mais fácil e rápida de determinar o sexo.

A evidência científica é sintetizada e avaliada através de revisões sistemáticas e meta-análises. Estas podem orientar a prática clínica, fornecendo informações sobre a eficácia dos tratamentos ou a precisão do diagnóstico e do prognóstico. No entanto, as relações entre variáveis individuais e resultados são o foco das revisões sistemáticas e meta-análises. Estas podem não captar todas as variáveis ou efeitos potencialmente relevantes em toda uma área de estudo. Os esforços em curso identificaram deficiências significativas na comunicação de sínteses narrativas, e a qualidade da realização e comunicação de revisões sistemáticas continua a ser de baixa qualidade. Os clínicos e os legisladores necessitam de orientações sobre a qualidade das revisões sistemáticas, mas a literatura existente não as fornece de forma adequada.

Palavras-Chave: Medicina Dentária Forense, Estimativa de sexo, Estimativa de idade, Revisão sistemática, Meta-análise, Umbrella Review.

ABSTRACT

Age estimation interest has grown in recent years, both because of the increasing number of unidentified corpses and because of the existence of living people without validated birth dates. There are two types of dental age estimation: developmental changes, which occur in human dentition during eruption, and degenerative changes, which occur during attrition. The eruption of the third molar occurs between the ages of 17 and 19 years. The tooth may be fully developed yet displaced, fully or partly displaced, or missing altogether. Only radiographs can provide accurate documentation of its status. Sex determination is important in forensic dentistry to help identify unknown persons. There are four ways to do this: skull analysis, DNA analysis of oral tissue, dental morphology, and measurement of mesiodistal and lingual vestibular dimensions, the latter being the easiest way to determine sex.

Evidence is synthesized and evaluated through systematic reviews and meta-analyses. They can guide clinical practice by providing information on the effectiveness of treatments or the diagnostic and prognostic accuracy. However, the relationships between individual exposures and outcomes are the focus of systematic reviews and meta-analyses. They may not capture all potentially relevant exposures or effects in a whole study area. Ongoing efforts have identified significant deficiencies in the reporting of narrative syntheses, and the quality of conducting and reporting systematic reviews continues to be low. Clinicians and policy makers need guidance on the quality of systematic reviews, but the existing literature does not provide it adequately.

Keywords: Forensic Dentistry, Sex estimation, Age estimation, Systematic review, Meta-analysis, Umbrella Review

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ABBREVIATIONS LIST

2D – Two dimensions

3D – Three dimensions

AI – Artificial Intelligence

AMSTAR - Assessment of Multiple Systematic Reviews

CBCT – Cone-beam computer tomography

CT – Computerized tomography

DNA - Deoxyribonucleic acid

I3M – 3rd molar Index

Ii – Incisor index

MDI – Mesiodistal Incisor diameter

MRI - Magnetic resonance imaging

PCR - Polymerase chain reaction

PRISMA – Preferred Reporting Items for Systematic reviews and Meta-Analyses

RCT – Randomized clinical trial

SD – Standard deviation

SRs – Systematic Reviews

SWiM - Synthesis without meta-analysis

TRIPOD - Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis

US – United States

CHAPTER 1

1. General Introduction

1.1. Forensic Dentistry

1.1.1. History

Forensic dentistry, also known as forensic odontology, involves the application of dental knowledge and techniques to legal matters. It involves the use of dental evidence to help identify human remains, determine the cause of death, and solve crimes (1–4).

The first recorded use of dental evidence in a criminal investigation was in China in 46 AD. A man was accused of biting his neighbor to death, and his teeth were compared to bite marks on the victim's body to establish guilt. In the 19th century, forensic dentistry began to be widely used in Europe and North America (5,6).

In 66 AD, well-documented evidence of the use of teeth for identification began with the cases of Agrippina and Lollia. Agrippina, after her marriage to Claudius, Emperor of Rome, feared that the wealthy divorcee Lollia Paulina might still be a rival for her marriage. She ordered her soldiers to kill Lollia Paulina and bring back the head. Lollia's identity has been established through dental alignments as well as certain distinctive features. It was the first use of dental identification where it is known to exist (7).

One of the earliest cases involved a Frenchman named Jean-Baptiste Troppmann, who was convicted of murdering the Kinck family in Paris in 1869. A dentist was able to identify one of the victims by matching her dental records to a set of dentures found in the river where the bodies had been dumped (5–7).

In Boston in 1776, Dr. Joseph Warren was killed at the Battle of Breed's Hill. He had suffered a fatal head wound and his face was unrecognizable. Paul Revere, a dentist, identified Dr. Warren's decomposing body by the small prosthesis he made for him. This identification allowed Dr. Warren to be buried with full military honors (8,9).

In the early 20th century, forensic dentistry became more sophisticated with the advent of X-rays. Dentists were able to use radiographs to identify individuals,

even when only partial remains were available (10). During World War II, forensic dentistry played a crucial role in identifying the remains of soldiers killed in action. The US Army Dental Corps established the first forensic dental team, responsible for identifying more than 90% of the remains recovered from the Pacific theater (11,12). In 1970, the American Board of Forensic Odontology was established to certify forensic dentists and promote the use of dental evidence in legal proceedings (5,6,13).

Today, forensic dentistry is a well-established discipline with a wide range of applications. It is used not only in criminal investigations, but also in disaster victim identification, civil litigation, and other areas where dental evidence can be relevant (14–17).

1.1.2. Importance

In today's world, crimes, human rights abuses, and natural disasters continue to occur. The number of missing and unaccounted people is increasing. The identification of living individuals or corpses is currently a particularly relevant area within forensic science. The individual right to identification is currently one of the most important universal rights (18,19).

Pelvic and skull bones can be used for more accurate identification. However, in extreme cases, such as natural disasters or mass graves, these bones are rarely in optimal condition, so they can't be used to make accurate estimates. However, teeth are the most resilient part of the human body. They not only survive after death, but remain unchanged for thousands of years, and are therefore considered quite useful in these scenarios, since they are often recovered intact (20–22).

Forensic dentistry was defined by Keiser-Neilson in 1970 as "that branch of forensic medicine concerned with the proper handling and examination of dental evidence and the proper evaluation and presentation of dental findings in the interest of justice" (3).

There are several contexts in which forensic dentistry can be used. As mentioned before, one of those is the identification of human remains. Therefore, even when other forms of identification are unavailable, dental records and radiographs (X-rays) can be used to identify a person's remains. Forensic dentists can also analyze bite marks on a victim's body or on an object found at a crime scene to help identify the offender, examine dental trauma (such as fracturing, chipping, or wear patterns) to determine the cause of the injury and whether the injury was caused by a criminal act, or provide expert testimony in cases of alleged dental negligence, such as inappropriate treatment or failure to diagnose a condition (2,23).

Forensic dentists work closely with law enforcement, coroners and other legal professionals to provide critical evidence in legal proceedings. They must have specialized training in forensic techniques and procedures, as well as a strong foundation in dental science. In addition, forensic dentists must be able to communicate complex dental information in a clear and effective manner to legal professionals and other members of the justice system (23,24).

The request for proof of identity of an object or person, which is a component of criminalistics, is the most common request made by the legal system to a forensic scientist. This forensic science involves linking an evidentiary item that is typically associated with a crime (24–26).

In criminal cases, the forensic dental examiner assists the judge or jury by providing expert testimony regarding a dental identification examination or the identification of bite marks and an opinion as to who may have made the bitemark (24–26).

Forensic identification involves two essential steps: The first step is to compare an unknown evidentiary item to a known item and have the forensic scientist make a judgment as to whether there is sufficient agreement to say that it is a "match" (24–26).

Dental identifications based on radiographs and dental records have generally been a reliable method and have rarely been challenged in court (26,27).

Dental examination and x-rays can also be used to estimate an individual's age or sex, which can be important in cases where the individual's documentation is unknown or the human remains cannot be directly identified (23,28,29).

1.1.3. Identification in Forensic Dentistry

Several identification methods have been used in forensic dentistry. The following are the various methods used in forensic dentistry:

Dental records contain detailed information about a person's teeth, including x-rays, photographs, and dental charts. By matching these dental records to a corpse, the person can be identified even if their skin and many other parts of their remains are unidentifiable. A bite mark can also be matched to a perpetrator using dental records (30,31).

Teeth can be used to identify a person based on their bite mark, which is the pattern the teeth make when they bite or occlude. Forensic dentists can compare the bite mark to the suspect's dental records to determine if they match. In cases of assault, sexual assault, and homicide, bite mark analysis can be crucial evidence (30,32,33).

A forensic dentist can extract DNA from the pulp chamber to crossmatch and identify a victim (29,30).

A forensic dentist can use ameloglyphics or enamel rod patterns. Similar to fingerprints, these are unique to each individual. Unlike fingerprints, they are highly resistant and cannot be intentionally burned or cut to alter the pattern. Like fingerprinting, examiners access the enamel (29,34).

Palatal rugae are the ridges on the roof of the mouth. They can be used for identification and are unique to each individual (29,35).

Cheiloscopy is the study of lip prints. Lip prints are unique to each individual and can be used for identification purposes (9,29,36).

Rugoscopy is the study of the palatal rugae. Palatal rugoscopy is unique to each individual and can be used for identification purposes (29,37).

To summarize, forensic dentistry uses various methods for identification, including dental records, bite mark analysis, DNA analysis, ameloglyphics, palatal rugae, cheiloscopy, and rugoscopy. These methods can be crucial evidence in criminal investigations and disaster victim identification and can provide valuable information about a person's identity.

1.1.3.1. Importance of dental identification in forensic dentistry

Dental identification is a critical aspect of forensic dentistry. It plays a central role in the identification of human remains of victims, not only those of mutilated, burned, and decomposed bodies, but also victims of bioterrorism and mass disasters. Catastrophic events have also underscored the importance of forensic odontologists in the identification of victims of industrial accidents, aviation accidents, natural disasters, and terrorist attacks, including those of explosive, chemical, radiological, or nuclear origin, and may occur as a single catastrophe or a widespread event (30,38).

Forensic dentistry plays a critical role in cases where common identification methods, such as fingerprinting and visual recognition, cannot be performed on decomposed, charred, or skeletal bodies. Dental records are an essential component of dental identification. Accurate maintenance of dental records is more common among dental students and dental schools. Therefore, private clinics should be instructed and encouraged to keep up-to-date dental records as a possible aid in crisis situations (31,33).

Dental records contain valuable information that can be useful to both dentists and legal authorities during forensic human identification. Dental records can

provide information about the individual's age, sex, race, and dental history, which can aid in the identification process (32,33).

Dental records can also narrow a person's search area and play a key role in the victim identification process following mass disasters or catastrophes. The dental identification process must be conducted carefully and relies on comparing information from the antemortem record with the postmortem record. The basic principles of dental identification are based on the uniqueness and identity of the deceased in forensic examinations. Dental characteristics can potentially lead to a positive identification in the same manner as DNA profiling and morphological fingerprints. However, these characteristics may not be of much help to the examiner in the absence of antemortem dental records (32,33,37).

In conclusion, dental identification is a crucial aspect of forensic dentistry. It plays a central role in the identification of human remains of victims, not only those of mutilated, burned and decomposed bodies, but also victims of bioterrorism and mass disasters. Dental records are essential for dental identification and contain valuable information that can be useful to both the dentist and legal authorities during forensic human identification. Dental identification must be performed with care and relies on comparing information from the antemortem record with the postmortem record. Dental characteristics can potentially lead to a positive identification in the same manner as DNA profiling and morphological fingerprints. However, these characteristics may not be of much help to the examiner in the absence of antemortem dental records.

1.1.3.2. Sex Determination in Forensic Dentistry

Dental identification plays a crucial role in sex determination in forensic dentistry. When human remains are found, especially in cases where the body is severely decomposed, burned, or fragmented, dental records can be invaluable for establishing the identity of the individual. Additionally, teeth are highly resistant to degradation and can withstand extreme conditions, making them reliable for identification purposes (20–22,29).

In terms of sex determination, teeth can provide important clues due to sexual dimorphism, which refers to the physical differences between males and females. Although there is significant individual variation, certain dental characteristics tend to be more prevalent in one sex than the other. By analyzing these features, forensic odontologists can reasonably assess the biological sex of an individual (39,40).

Some features are tooth size, in general, males tend to have larger teeth than to females. Measurements of tooth dimensions, such as crown and root sizes, can be helpful in determining the sex. Canines often exhibit sexual dimorphism. In males, these teeth are typically larger and more robust, with a greater mesiodistal width, whereas females tend to have smaller and more rounded canines. Males generally have wider interdental spaces, especially in the posterior region, whereas females often have narrower spaces. Additionally, the shape of the dental arch can provide clues about sex. Males often have broader and more square-shaped arches, while females tend to have narrower and more U-shaped arches. Regarding patterns of dental wear, it can differ between sexes, males may exhibit more pronounced wear on the incisal edges of anterior teeth due to habits like pipe smoking or chewing on hard objects. Only a small number of studies have used skeletal remains, probably due to the difficulty of obtaining a large sample of intact bodies with a complete set of teeth (21,29,41).

It is important to note that dental identification is most effective when combined with other methods, such as DNA analysis, skeletal examination, and anthropological assessment. Moreover, the accuracy of sex determination based on dental features depends on the quality and availability of comparative dental records and the expertise of the forensic odontologist conducting the analysis (29,42,43).

Overall, dental identification, including the assessment of dental characteristics related to sex determination, can provide valuable information in forensic investigations, aiding in the identification and profiling of human remains.

1.1.3.3. Age Determination in Forensic Dentistry

Dental age determination is an essential factor in human identification, forensic investigations, and clinical dentistry. Teeth are bestowed with features such as hardness and resilience to withstand the test of time, making them an excellent tool for age estimation. The process of dental age determination involves the examination of teeth and their surrounding structures, including the alveolar bone, pulp cavity, and periodontal ligament. Several methods have been developed for dental age determination, including radiographic and non-radiographic methods (22,29,44,45)

Radiographic methods are the most used techniques for dental age determination. These methods involve the use of dental radiographs to examine teeth and their surrounding structures. The most used radiographic methods are the Demirjian, Willems, and Haavikko methods. These methods use the development and eruption of teeth to estimate age (29,46,47).

Non-radiographic methods for dental age determination include the use of tooth wear, tooth cementum annulation, and tooth translucency. Tooth wear is the loss of tooth structure due to attrition, abrasion, and erosion. Tooth wear can be used to estimate age, as it is a gradual process that occurs over time. Tooth cementum annulation is the formation of rings in the cementum of the tooth root. These rings can be counted to estimate age, as they are formed annually. Tooth translucency is the degree of transparency of the tooth enamel. The degree of translucency increases with age, and this can be used to estimate age (17,45,46).

Although these various dental age estimation methods have been confirmed for children and adolescents, there are few reliable methods with low error rates and high reliability for adults and the elderly. In addition, these conventional methods focus on one or two characteristics in panoramic radiographic images, and do not consider other parts of the images except for the parts with attention. However, recent studies have shown that artificial intelligence (AI) can be used to estimate dental age with high accuracy. AI-based methods use machine learning algorithms to analyze dental images and estimate age. These methods have

shown promising results and have the potential to revolutionize dental age determination (42,48).

In conclusion, dental age determination is an essential tool in forensic investigations, clinical dentistry, and human identification. Radiographic and non-radiographic methods have been developed for dental age determination, with radiographic methods being the most used. Recent studies have shown that AI-based methods can be used to estimate dental age with high accuracy and have the potential to revolutionize dental age determination. Further research is needed to develop reliable methods for dental age determination in adults and the elderly.

1.1.3.4. Sex Determination Procedures in Forensic Dentistry

By excluding approximately half of the population, accurate sex prediction is a critical step in creation of a postmortem forensic profile. Several studies have shown that teeth exhibit a high degree of sexual dimorphism. In general, male teeth are larger than female teeth, but the data are not consistent and there is also reverse dimorphism. The degree of sexual dimorphism may vary between different populations, possibly due to differences in the environment, available food resources or the genetic pool (21,39,45).

Among the methods of estimating sex through metric analysis, we highlight those that use the mesiodistal distance of the teeth, those that calculate the volume of the pulp chamber as a criterion of sexual dimorphism, those that work with the Mandibular Canine Index, and those that aim at three-dimensional geometric analysis to distinguish human sex. Odontometric methods are based on the study of tooth measurements using calibrated measurement instruments, such as a caliper. Due to the subjectivity of the analysis based on the external appearance of the tooth, this method is more reliable than morphological techniques (29,49).

Capitaneanu et al. (29) suggested that any reliable method should have an accuracy greater than 80% and that if the first search for a positive identification is negative, the second search should follow for both sexes. However, many

studies have focused on a young adult population in order to determine the presence of healthy teeth and to remove factors such as tooth wear, tooth decay and tooth fractures from the dental measurements, which might create a bias (29,39,45,50).

With regard to sex determination using canine dimorphism, permanent canines and their arch width (distance between canine tips) have been studied using several methods, particularly the measurement of linear dimensions such as m-d width, buccolingual width, and inciso-cervical height. As mentioned above, the mandibular canine width and the intercanine distance were greater in males than in females and the correct sex classification ranged from 74% to 88% (29,39,50,51).

There is controversy regarding the degree of sexual dimorphism between maxillary and mandibular canines in different ethnic groups. Therefore, this method may be inadequate as a sole tool for forensic sex determination because it may not be possible to establish a single value that can be applied to all populations (29,51–53).

In addition to absolute tooth size, tooth ratio has been proposed to differentiate between the sexes, e.g., the 'incisor index' (Ii), which is calculated by the formula Ii = (MDI2/MDI1) x 100, where MDI2is the largest diameter of the upper lateral incisors and MDI1is the largest diameter of the central incisors. This index tends to be higher in men, suggesting that the lateral incisors are significantly smaller than the central incisors in women (32,41).

Another index that provides a relatively accurate indication of sex is the "mandibular canine index". Using the mandibular canine's mesiodistal dimension and the following formula: ([mean mesiodistal canine dimension + [mean mesiodistal canine dimension in females + standard deviation (SD)] in males - SD])/2, we obtained a value. When the value is lower than 7.1, the subject is female and when it is higher, the subject is male (9,50). Sex determination success rate using the above formula was nearly 89% (50,51).

The robustness index and the crown module of the canine are two other odontometrics that show significant differences between males and females. The robustness index is calculated by the formula, mesiodistal distance x buccal-lingual distance; and the crown module by the formula (mesiodistal distance x buccal-lingual distance)/2. In both cases, males showed significantly higher values than females (41,54).

Finally, discriminant function analysis is the most popular statistical model for assessing tooth sex. Discriminant analysis is more reliable in differentiating sex than comparing teeth independently, as teeth are considered as a unit (multivariate analysis). More recently, logistic regression analysis was considered a better alternative, and its application was found to be superior to discriminant analysis (~45-73%) in terms of correct sex assignment rate (76-100%). When all teeth from both jaws were included, logistic regression analysis provided optimal sex prediction (21,22,55,56).

1.1.3.5. Age Determination Procedures in Forensic Dentistry

There are several strategies for estimating dental age, depending on whether tooth development (around 20 years of age) or body development is complete. On the one hand, methods that are based on the development of the teeth are more accurate and have a smaller margin of error (46,47). On the other hand, the biological age of the individual will always be estimated in terms of a period of time, with a certain degree of precision and in accordance with the method in use. At best, chronological age is incorporated into this age range (45,46).

Several methods have been developed; however, dental measurements and indices are regarded as the most useful and reliable because they are less variable and more resistant against confounding factors (57,58). Most studies have used 2D radiographs, such as the Demirjian and Willems methods, in different populations worldwide. Both tend to overestimate at different levels. Cameriere's method has been studied (28,59–61), first for several age groups and later as an index (I3M) that differentiates the legal threshold of 18 years. Other methods include those of Chaillet, Lundbergs, Nolla, Mornstad, Haavik and

Kval (59,61,62). Recently, a small number of studies have investigated the applicability of three-dimensional imaging in the field of forensic dentistry (61,63,64).

Based on a sample of French-Canadian children, Demirjian's method (65) was developed in 1973 and revised in 1976. This method involves the evaluation of eight specific stages of tooth development in third molars only (59,66–70). This method has gained worldwide acceptance and has become the most widely used method for estimating the dental age estimation (66). However, most studies (66–68,70) concluded that this method is geographically sensitive and varies depending on the subpopulation studied. A possible reason for this is the origin of this dataset (Caucasian subpopulation) with low heterogeneity, which is different from the other subpopulations studied (66–68,70). Regardless of the sex of the subject, Demirjian's method tends to overestimate the age of the individuals. Hostuic et al. (67) performed an analysis to validate this claim, separating studies by ethnicity and obtaining different results, although they also note that these results tend to blur in the mixed genetic pool that most countries' populations are today. Jayaraman et al. (68) disagree with most of the studies, attributing the differences to methodological errors rather than ethnic differences. As for sex differences, the studies do not agree, with some overestimating men (67), others overestimating women (66,68), or even finding that sex is not a cofactor (59,69,70). Demirjian's method only goes up to 16 years of age. Therefore, it becomes uncertain and questionable in more advanced age groups. Moreover, the lack of bilateral mandibular teeth makes it impossible to use this method. Therefore, the Demirjian procedure is a poor evidentiary tool if applied incorrectly (67,68). In 1993, Mincer et al. (71) proposed Demirjian's developmental stages for third molars, extending the estimated age range to 25 years. It has been applied in all continents except Oceania. Ethnic differences appear to be smaller when applied to the 3rd molar (72,73). Sex also seems to have little or no influence when considering the developmental stages of the 3rd molar (72,73).

Willems' method is a modification of Demirjian's method introduced in 2001 (47), based on a Belgian population, with a different conversion table from dental

maturation score to dental age. Although most of the studies were conducted on Asian populations, all five continents are represented by some kind of subpopulation (59,66,69,74–76). Four studies evaluating different subpopulations concluded that geographic profile affects outcomes (66,74–76). For instance, Sehrawat et al. (75) found that this method overestimates in most countries, with the exception of China and India. This conclusion is consistent with Prasad et al. (69), who analyzed only the Indian subpopulation and found an underestimation of approximately 1 month. This variation tends to reinforce the general idea that tooth development is influenced by external factors such as nutrition, environment, and endemic diseases, as well as genetic factors (66,74–76). Another reason for this variation is that this method was developed on a Caucasian subpopulation with low heterogeneity, which is not the case for some of the subpopulations analyzed (76). Sex appears to be a factor to consider, as this method tends to overestimate males more than females (66,69,74–76).
Three studies comparing the Demirjian and Willems methods agreed that the latter is more accurate and less prone to overestimation (59,66,69).

Cameriere's method was published in 2006 and is based on measuring the ratio of the distance between the inner sides of the open apex divided by the tooth length (77). It was originally applied to single rooted teeth and later to the rest of the dentition up to the 2nd molar. It is one of the most geographically stable methods and tends to overestimate by 4 months in both boys and girls, with no statistically significant difference (59–61). There is also no statistically significant difference between performing this method on the right or left lower quadrant (61). Marroquin et al. (61) reported an overestimation of 10 years in an Indian subpopulation, but only when Cameriere's method was used in an adult group, which tended to increase the margin of error for dental age estimation methods (61). According to Hostiuc et al. (2021) (60) this method appears to outperform several others, including the Dejirmijian and Willems methods (60).

In 2008, Cameriere proposed an index (I3M) based on the same method mentioned above but related to the 3rd molar, defining a threshold to differentiate a legal landmark of majority, being younger or older than 18 years (14). Santiago et al. (28) concluded that the I3M has been validated worldwide in different

subpopulations, because it has a high accuracy in discriminating if an individual has reached 18 years of age, regardless of the population studied. Regarding sex, a trend of better results was observed for males, but high values of accuracy, sensitivity, and specificity were also achieved for females (28).

Chaillet's method is based on Demirjian's method, which uses new data from eight different subpopulations. He also added the third molar to the evaluation and two additional maturity scores, allowing this method to estimate age beyond 16 years, similar to the original Demirjian method (62). As previously reported, this method has a tendency to overestimate by approximately 6 months in most geographic subpopulations, with a greater overestimation in the Asian population. This wider range of overestimation with wider confidence intervals can be explained by the possibly greater heterogeneity of the pooled data used to develop the Chaillet method (62).

Kvall's method, published in 1995, uses periapical radiographs to measure the maximum tooth length, the root length on the mesial surface, the maximum pulp length, the root length on the mesial surface, maximum pulp length and tooth width at three different levels (61). Later in 2005, this method was applied to panoramic radiographs. Studies that obtained more accurate results. After full maturation in the 3rd decade of life, this method seems to have an average error of 20 years. It is not agreed that Kvall's method is affected by the sex of the individual, although the original study although the original study included it as a factor (61).

The remaining methods (Nolla, Lilequist and Lundberg, Mornstad and Haavikko) were only applied to a Brazilian subpopulation, so these results must be interpreted with caution. With the exception of Nolla, the other methods are not widely used in dental age estimation studies and lack geographically specific validation to verify their usefulness and applicability (59).

3-D imaging is becoming increasingly important in all areas of dentistry, including forensic dentistry, with the first study reporting the use of this technology published in 2004 (78). CBCT and CT scan reconstructions allow investigators to

analyze the pulp/tooth volume ratio. The volume ratio could be an interesting tool for predicting age after root maturation of the 3rd molar, around the second decade of life (63). Although micro-CT scans require the use of extracted teeth, the images are of higher quality and provide accurate measurements due to the higher spatial resolution than CBCT. However, its application in living subjects is not feasible, and a model based on this type of imaging may not be reproducible for forensic proposals in living individuals (61,63). A major limitation of this method are the artifacts caused by adjacent metal structures and restorations, such as implants and amalgam fillings (63). Also, the difficulty in reproducing the site for measurements could lead to inaccurate analysis; the lack of a simple method of examination should be the focus of future research (63).

Due to the ethical implications of using ionized radiation for other than diagnostic indications, MRI has emerged as a valid alternative, as it uses strong magnetic fields and radio waves to generate images. It is a relatively new tool for age estimation, first published in 2015 (79). As for the method itself, MRI can be used in conjunction with other previously mentioned methods, such as Demirjian's method. MRI tends to be more accurate in the annual stages of development, but it is more difficult and inaccurate in the later stages due to the lack of contract between dental and bone tissue. It also takes more time and is more expensive than a standard panoramic radiograph (63). Due to the lack of research on this topic, the interethnic variability is not yet proven (64). The differences between MRI approaches make it inappropriate to pool data and perform a proper systematic review with meta-analysis. In addition, future MRI-based age estimation methods are likely to be based on multifactorial sites and measures (64).

1.2. Methodologies for systematic reviews validation

In the healthcare field, systematic reviews and meta-analyses are becoming increasingly important. It is now more important than ever for the clinician to stay up to date with the latest findings and advances in health care. However, as more studies are published and journals continue to proliferate, it can become difficult for busy clinicians to remain adequately informed (80–82).

Systematic reviews and meta-analyses are syntheses and evaluations of the evidence to provide information on the effectiveness of treatments or the accuracy of diagnostic and prognostic procedures that can guide clinical practice (82–84).

Systematic reviews are a thorough examination and synthesis of research on a specific topic regarding a clinical problem. It has a specific methodology which makes it different from a general audit. The homogeneity or heterogeneity of different study methods and designs should be captured in systematic reviews. The conclusions of the review should be drawn from studies that address the same research question. A simple comparison of the number of positive studies with the number of negative studies is not enough. Systematic reviews include an assessment of the quality of the different studies and a weighting of the studies. Larger studies and randomized controlled trials are given more weight. The development of an RCT or clinical guideline on a topic is often preceded by a systematic review. The gold standard for systematic reviews worldwide is the Cochrane Collaboration. The Cochrane Collaboration is a global network of health care professionals, researchers, patient advocates, and others who produce high-quality systematic reviews, syntheses, and recommendations from these in-depth reviews (81,84–86).

A meta-analysis is a systematic review that uses statistical techniques to combine and summarize the quantitative results of similarly designed studies. This method allows the estimation of the magnitude of the effect of an intervention or risk factor and subgroup analysis by combining the results of many studies. Meta-analyses require a high degree of homogeneity among the studies examined with respect to design, methodology, and reporting of data. Meta-analysis is a narrow presentation of relevant research on a given topic, designed to provide more precise information about the direction of a positive or negative effect (87,88).

A systematic review, which methodically selects and appraises clinical trials, provides a synthesized view of the best available evidence to answer a specific research question or set of questions. It also highlights the quality of the evidence and gaps in knowledge. These benefits place systematic reviews at a higher level

in the evidence-based hierarchy. A meta-analysis is often conducted in conjunction with a systematic review. A meta-analysis provides a more precise answer to a specific clinical question by quantitatively combining data from individual studies. Consuming information from well-conducted and well-reported systematic reviews is one-way clinicians can keep up with the scientific evidence without reading numerous research articles on the subject (82,84,88).

However, systematic reviews and meta-analyses tend to focus on the relationships between individual exposures and outcomes, which may not capture all the potentially related exposures or outcomes across an entire field of study. In addition, the increasing number of overlapping and conflicting reviews has been a continuing concern. These limitations highlight the need for a study design that has the potential to provide a higher level of synthesis of the evidence at the summary level. As with other publications, the quality of reporting in systematic reviews is variable, which limits the reader's ability to assess the strengths and weaknesses of these reviews (80,83,86).

Umbrella reviews summarize the extent and strength of associations reported in previously conducted systematic reviews and meta-analyses, also known as overviews of systematic reviews or systematic reviews of meta-analyses. They can take into account multiple exposures and outcomes; provide an assessment of the effect of sample size, heterogeneity, and evidence of bias on the summary associations; and assess the quality of individual systematic reviews and meta-analyses. These assessments are particularly useful in areas where many reviews have already been conducted and have grown in popularity over the past decade (80,83–86).

1.2.1. PRISMA (Preferred Reporting Items For Systematic reviews and Meta-Analyses)

Although progress has been made, the quality of the conduct and reporting of systematic reviews remains far from ideal. There is considerable evidence that the reporting of key information in systematic reviews is often poor, and that their potential usefulness is therefore limited. To enable readers to assess the

strengths and weaknesses of the research, systematic reviews should be reported fully and transparently (81,82,89).

Published in 2009, the PRISMA statement was developed by a group of 29 review authors, methodologists, clinicians, medical editors, and consumers who created a 27-item checklist and a four-phase flowchart. PRISMA's overarching goal is to provide clarity and transparency in systematic review reporting, and recent data suggest a strong need for such reporting guidelines. PRISMA is not designed to be, and should not be, a quality evaluation tool, but it may benefit systematic reviewers, potentially reducing the risk of too many reviews addressing the same question and making the updating of systematic reviews more transparent (81,82,89).

The focus of PRISMA was on randomized trials, but PRISMA can also be used as a basis for the reporting of systematic reviews of other types of research, especially evaluations of interventions. Items on the checklist were included when there was evidence that failure to report the item was linked to an increased risk of bias, or when it was clear that the information was needed to assess the reliability of a review (81,82,89).

PRISMA is a living document, as is any evidence-based endeavor. Maintaining PRISMA as up-to-date and evidence informed as possible requires regular monitoring of the fast-growing literature. PRISMA's recommendations are widely supported and adopted, as demonstrated by their being co-published in multiple journals, cited in more than 60,000 reports, endorsed by nearly 200 journals and systematic review bodies, and adopted across multiple specialties (81,82,89).

1.2.2. SWiM (Synthesis without meta-analysis

Serious deficiencies in the reporting of narrative syntheses have been identified in recent work. These include failing to describe the methods used, failing to provide clear links between the study data and the text in which the synthesis and its conclusions are reported, and failing to adequately report the synthesis's limitations (90,91).

An estimated 32% of systematic reviews of health interventions do not use meta-analyses, but instead use alternative approaches to synthesis, usually based on textual description of effects, often referred to as narrative synthesis (90).

This indicates widespread unfamiliarity with and misunderstanding of the requirements for transparent reporting of synthesis in the absence of meta-analyses, and points to the need for reporting guidance (90).

PRISMA provides only limited guidance on how to report on certain aspects of the review, such as the methods of presentation and synthesis, and there are no reporting guidelines for the synthesis of effect estimates without a meta-analysis (81,82,89).

The SWiM reporting guideline aims to facilitate transparent reporting of effect estimate synthesis without a meta-analysis. The guideline is likely to be most relevant to reviews that include multiple data sources that are not amenable to meta-analysis, and specifically addresses transparent reporting of synthesis and presentation methods and results. It is recommended that SWiM to be used together with other reporting guidance covering other aspects of conducting reviews, such as PRISMA (81,82,89,91). The SWiM guideline is not intended to be used as the sole basis for the presentation of a review; such situations may arise when the reporting of effect estimates is incomplete, or when the characteristics of the studies (like study designs, types of interventions, or outcomes) are too diverse to produce a meaningful summary effect estimate. Such reviews may use alternative methods of presentation and synthesis (such as calculating summary statistics of intervention effect estimates, counting votes based on direction of effect, and combining p-values), and SWiM provides guidance on reporting these methods and results (90,91).

SWiM covers reporting key features of the synthesis, including grouping of studies, synthesis methods, data presentation and summary text, and synthesis limitations. They have identified nine elements to guide the reporting of non-meta-analysis syntheses. These include reporting the structure of the synthesis and the comparison groups, the standardized metric used to conduct the synthesis,

the method of synthesis, the presentation of the data, and a summary of the results of the synthesis that is clearly linked to the supporting data. SWiM promotes the basic principles necessary for transparent reporting of all synthesis methods, including meta-analysis, although the SWiM items were developed specifically for the many reviews that do not include meta-analysis. Thus, the SWiM elements have relevance for reporting the synthesis of quantitative outcomes data, regardless of the technique employed (90,91).

As such, SWiM is intended to be a resource for review authors and to assist editors and readers of journals in assessing the conduct of a review and the validity of its findings (90).

1.2.3. AMSTAR (Assessment of Multiple Systematic Reviews)

Systematic reviews are complex endeavors and assessing their quality can be a formidable challenge. Guidance on the quality of systematic reviews is needed by clinicians and policy makers but is not adequately provided in the existing literature. Existing quality assessment tools and instruments have been less than optimal and need to be revised and updated (83,85,92).

This leads to confusion for those who use reviews in clinical and policy decision-making and who need to be able to distinguish between reviews of good quality and those of poor quality. Therefore, there is a need for a reliable and valid quality assessment tool that is easy to use (83,85).

Based on this assessment, the Assessment of Multiple Systematic Reviews (AMSTAR), a new instrument for the assessment of systematic reviews, was created in 2007 and updated in 2017. AMSTAR is reliable, valid, and easy to use. It was designed to build on and update the best of the published literature, rather than being a truly original instrument (83,85,92).

AMSTAR has been tested only in systematic reviews of randomized and/or non-randomized trials; however it can be applied to a wide variety of systematic reviews. It can be scored on an individual basis (components) and as a checklist

by summing the scores of the individual items (total score). It has been developed psychometrically so that each item can be scored as if it were independent of the others. Each of the components was found to be separate in the factor analysis. Therefore, all assessments have an equal chance of scoring well. However, the meta-analysis will score slightly higher in the overall results (83,85,92).

AMSTAR 2 can be used as a short teaching tool or checklist by those conducting systematic reviews, as it is structured around the main sequential steps in conducting a systematic review. The AMSTAR instrument consists of an 11-item questionnaire, with each item scored 1 if the specific criterion is met, or 0 if the information is not provided, not clear, or the criterion does not apply. The scores for each item were averaged across the five raters when reviewing the evidence for each guideline. The average scores for all 11 items were added to calculate the total AMSTAR score for each evidence review (83,92).

Methodologically, it is worthwhile to evaluate the component ratings because they measure different items, and some may be more important than others in certain settings. Thus, a summary score can mask important strengths or weaknesses. In the case of AMSTAR, an attempt has been made to develop a pragmatic tool of value to decision makers. It is valid and easy to use, and the overall score is meaningful. This means that the total score has been separately validated against an external standard, and that the components have been ensured not to overlap. For example, it is important to assess this aspect of your conduct because funding sources are associated with bias in systematic reviews (83,85).

Considering that the risk of bias in individual studies is equally important for randomized and nonrandomized studies of health interventions, it is usually easier to understand the former. Increasingly, the real-world impact of a wide range of health technologies and practices is being assessed using large non-randomized studies, usually conducted on large administrative databases. Even though such studies are often well-designed, the remaining confounders or other sources of bias may result in inaccurate estimates of impact. Meta-analyses can produce accurate but biased estimates of intervention effects by including large observational studies (83,85).

CHAPTER 2

2. Aim

The need for age estimation has increased in recent years due to an increase in the number of unidentified cadavers and the existence of living individuals who have no valid proof of birth date. The assessment of dental aging can be divided into two categories: developmental changes that occur in the human dentition during tooth eruption and degenerative changes that occur during tooth wear. The third molar erupts between the ages of 17 and 19. This tooth may be fully developed but impacted, fully or partially impacted, or completely absent, and only radiographs can accurately document its status. On the other hand, sex determination is an area of great importance in forensic dentistry for the identification of unknown persons. This can be done by four methods: cranial analysis, DNA analysis from oral tissues, dental morphology, and measurement of mesiodistal and lingual vestibular dimensions, the latter being the simplest method for sex determination.

Systematic reviews and meta-analyses are used to synthesize and evaluate evidence. They provide information about treatment effectiveness or diagnostic and prognostic accuracy that can guide clinical practice. Nonetheless, systematic reviews and meta-analyses tend to focus on the relationships between individual exposures and outcomes, which may not capture all potentially related exposures or outcomes across an entire field of study. Current work has identified serious shortcomings in reporting narrative syntheses, and the quality in conducting and reporting systematic reviews remains poor. Clinicians and policy-makers need, but the existing literature does not adequately provide, guidance on the quality of systematic reviews.

Therefore, the main aim of this thesis was to development and validation of a sex and age prediction model considering dental measurements. The second aim was to assess the level and quality of previously published literature regarding the determination and prediction of sex and age in forensic dentistry.

To achieve these purposes, our specific aims were divided in two stages:

Stage 1 – Sex and age prediction models considering dental measurements.

1.1. To develop a new sex prediction model based on mesiodistal width measures (Paper 1);

1.2. To test the validity of the I3M in a Portuguese representative sample, by using panoramic radiographs. Secondly, we looked for the influence of sex on its validity (Paper 2).

Stage 2 – Assessment of the level and quality of the previously published literature

2.1. To perform an Umbrella Review of Sex Determination Procedures (Paper 3);

2.2. To perform an Umbrella Review of Age Estimation Methods (Paper 4).

CHAPTER 3

3. Sex And Age Prediction Models Considering Dental Measurements

This chapter was based on the published work:

Paper I – João Albernaz Neves, Nathalie Antunes-Ferreira, Vanessa Machado, João Botelho, Luís Proença, Alexandre Quintas, José João Mendes, Ana Sintra Delgado. Sex Prediction Based on Mesiodistal Width Data in the Portuguese Population" *Applied Sciences* **2020** 10, no. 12: 4156. 10.3390/app10124156

Paper II – João Albernaz Neves, Nathalie Antunes-Ferreira, Vanessa Machado, João Botelho, Luís Proença, Alexandre Quintas, Ana Sintra Delgado, José João Mendes, Roberto Cameriere. Validation of the Third Molar Maturation Index (I3M) to assess the legal adult age in the Portuguese population. Sci Rep **2020** 10, 18466. 10.1038/s41598-020-75324-x

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3.1. Sex prediction based on mesiodistal width data in the Portuguese population.

applied sciences

MDPI

Article

Sex Prediction Based on Mesiodistal Width Data in the Portuguese Population

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Received: 21 May 2020; Accepted: 13 June 2020; Published: 17 June 2020

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Abstract: Accurate sex prediction is a key step in creating a postmortem forensic profile as it excludes approximately half the population. It is our goal to develop a predictive model to establish sex through teeth mesiodistal widths in a Portuguese population. The pretreatment dental casts of 168 of Portuguese orthodontics subjects (59 males and 109 females) were included. Mesiodistal widths from right first molar to left first molar were measured on each pretreatment cast to the nearest 0.01 mm using a digital caliper. Overall, the mesiodistal widths of the upper and lower canines, premolars, and molars were found to be significantly different between females and males. Conversely, no significant differences between sexes were identified for incisors. A multivariate logistic regression model for sex prediction was developed and the teeth included in the final reduced model being the upper left canine (2.3), the lower right lateral incisor (4.2) and the lower right canine (4.3). There is a prevalence of sexual dimorphism in all teeth except the incisors. The canines present the most noticeable difference between sexes. The presented sex determination predictive model exhibits an overall correct classification of 75%, outperforming all available models for this purpose and therefore is a potential tool for forensic analysis in this population.

Keywords: forensic dentistry; sex determination; sexual dimorphism; dental measurements; predictive model; Portuguese population

1. Introduction

Forensic dentistry emerges as a part of forensic medicine and dental anthropology. This is the branch of dentistry that focuses on the issues of identifying human remains by direct comparison, bite mark identification, clinical malpractice, and forensic dental profiling, such as sex and age estimation, in cases of unknown human remains, in order to facilitate their subsequent identification [1,2].

Teeth are the hardest organ in the human body and very important in postmortem identification procedures. Although pelvic and cranial bones can be more accurate in identifying sex, they are rarely in optimal condition in extreme cases, such as natural disasters or mass graves, which may prevent accurate estimation through them. Teeth are considered quite useful in these scenarios as they are often recovered intact [3–6]. However, there may be some setbacks that prevent dental eruption of teeth useful for forensic identification [7–10].

Accurate sex prediction is a key step in creating a postmortem forensic profile as it excludes approximately half the population [11]. Several studies state that teeth have a high degree of sexual dimorphism [2,4,5,12,13]. Generally, male teeth are larger than female teeth, however data are not consensual and reverse dimorphism also occurs [4,12]. Sexual dimorphism may vary between different populations, possibly due to variations in the environment, available food resources, or genetic pool [3,14].

The most usual way to obtain data is from dental casts using a digital caliper. There are several measures to take into account and their analysis may be performed through direct comparison of measures, statistical analyses, or indexes.

Only two previous studies presented potential predictive sex models for Portuguese populations using dental measurements. Pereira et al. (2010) [1], using upper canine-to-canine teeth, rendered a combination of incisors mesiodistal and canine diagonal distances. As the proposed model was confined to only six teeth, it lacks a complete teeth analysis. On the other hand, Silva et al. (2015) [12] employed the mandibular canine index [15] with a modest success rate of 64.2%, concluding that this index should be restrictively applied to the Portuguese scenario in sex identification.

Given the lack of robust sex identification models for the Portuguese population, we aimed to use cast models of a previously studied sample (Machado et al., 2018) [16] to develop a new sex prediction model based on mesiodistal width measures. We hypothesize that there is a sex-based teeth dimorphism in this population and it is distinguishable through a predictive model; therefore our null hypothesis is that such dimorphism may not exist.

2. Materials and Methods

2.1. Study Design

This study used a previously reported sample [16] that has received approval by the Egas Moniz Ethics Committee (Number 600). Written informed consents were obtained from all participants during their first appointment at the Orthodontic Department of the Egas Moniz Dental Clinic.

This investigation follows the transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) reporting guidelines [17] for validation of prediction models (see supplementary material). This study was conducted on a triple-blind basis with respect to diagnosis and clinical outcome, data collection, and analysis.

2.2. Participants

The assessment tool consisted of pre-treatment dental casts, a part of standard orthodontic treatment planning, in dental stone selected from the archives of the Egas Moniz Dental Clinic Orthodontic Department (Almada, Portugal). From a total of 541 casts gathered from November 2010 to December 2017, 168 (59 males and 109 females) were selected according to the inclusion and exclusion criteria.

The inclusion criteria were: all teeth, from first molar in the right side to first molar in the left side in both upper and lower jaws, were fully erupted and present; no history of interproximal stripping; no proximal caries that might interfere with precise tooth measurement; no restorations, abrasions or attrition; no previous or ongoing orthodontic treatment; no abnormal tooth morphology or congenitally missing or impacted [16]. All patients failing to fulfil these criteria were excluded.

2.3. Dental Casts Analysis and Measurement Reproducibility

Dental cast measurements were performed by one researcher (VM) using a digital caliper to measure the mesiodistal tooth widths from the right first molar to the left first molar to the nearest 0.01 mm. The mesiodistal width of each tooth was measured at the widest distance between the mesial and distal contact points. The position of the caliper had to be perpendicular to the occlusal surface of the measured tooth [1,11,14,16,18–21].

Ten study casts were randomly chosen from the total of 168 and remeasured one week later by the same investigator (V.M.). Intraclass correlation coefficient (ICC) was calculated with an absolute agreement of ICC = 0.98.

2.4. Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 25.0 for Windows (Armonk, NY: IBM Corp.). Descriptive statistics as mean and standard deviation (SD) were determined for the mesiodistal width per tooth. Mean mesiodistal width for each tooth was compared according to sex and by Student's *t*-test. A multivariate stepwise adjusted logistic regression procedure was applied to derive a reduced predictive model for sex determination based on the mesiodistal widths for each tooth. To test the performance of the obtained model and compare it to previous ones, the sensitivity, specificity, accuracy, and precision were determined for all models when applied to the studied sample [22]. Performance measurement was assessed by binary area under the curve (AUC) and through receiver operating characteristics (ROC) analysis. The level of significance was set at 5%, in all statistical inference analyses.

3. Results

3.1. Mesiodistal Width Per Tooth

In the studied sample the mean age was 20.1 (±7.3) (see Machado et al., 2018 [16] for a detailed description). The mean mesiodistal width for each tooth, according to sex, is presented in Table 1. Overall, the mean mesiodistal widths of upper and lower canines, premolars, and molars were found to be significantly different between females and males. Regarding the incisors, no significant differences were found as a function of sex.

Table 1. Descriptive values of mesiodistal width (mm), presented as mean and standard deviation (SD) for each tooth, as a function of sex (n = 168).

Semi-Arch	Tooth Type	Tooth Code	Female (n = 109)		Male (n = 59)		*p*-Value *
			Mean (±SD)	Range (Min–Max)	Mean (±SD)	Range (Min–Max)	
Upper right	Molar	1.6	10.13 (±0.56)	8.50–12.52	10.40 (±0.60)	9.26–12.37	**0.004**
	Premolars	1.5	6.62 (±0.47)	5.51–8.04	6.40 (±0.52)	5.71–8.30	**0.008**
		1.4	6.93 (±0.47)	5.70–8.17	7.18 (±0.48)	6.35–8.50	**0.001**
	Canine	1.3	7.67 (±0.46)	6.40–8.90	7.97 (±0.57)	6.10–9.22	**<0.001**
	Incisors	1.2	6.64 (±0.61)	5.20–7.89	6.70 (±0.63)	5.25–8.55	0.565
		1.1	8.60 (±0.58)	7.00–10.05	8.68 (±0.66)	6.47–10.78	0.414
Upper left		2.1	8.62 (±0.54)	7.39–9.88	8.69 (±0.67)	6.47–10.66	0.437
		2.2	6.54 (±0.60)	4.73–7.89	6.71 (±0.65)	5.25–8.30	0.091
	Canine	2.3	7.65 (±0.46)	6.40–8.70	8.04 (±0.52)	6.83–9.22	**<0.001**
	Premolars	2.4	6.92 (±0.48)	5.70–8.17	7.17 (±0.50)	5.96–8.28	**0.002**
		2.5	6.62 (±0.47)	5.51–8.04	6.84 (±0.52)	5.70–8.30	**0.005**
	Molar	2.6	10.12 (±0.56)	8.50–12.52	10.33 (±0.59)	9.4–12.44	**0.018**
Lower right	Molar	4.6	10.63 (±0.61)	9.10–12.32	11.03 (±0.57)	9.82–12.17	**<0.001**
	Premolars	4.5	7.19 (±0.52)	6.04–8.93	7.36 (±0.54)	6.17–8.98	**0.050**
		4.4	7.08 (±0.43)	6.11–8.30	7.36 (±0.51)	6.22–8.50	**0.001**
	Canine	4.3	6.63 (±0.42)	5.84–8.15	7.01 (±0.48)	6.03–8.91	**<0.001**
	Incisors	4.2	5.89 (±0.43)	4.50–6.94	5.97 (±0.44)	4.98–6.95	0.253
		4.1	5.38 (±0.43)	4.50–7.60	5.46 (±0.43)	4.68–6.51	0.280
Lower left		3.1	5.38 (±0.44)	4.50–7.60	5.41 (±0.47)	3.73–6.37	0.708
		3.2	5.86 (±0.44)	4.50–6.78	5.99 (±0.42)	4.92–6.99	0.052
	Canine	3.3	6.63 (±0.41)	5.84–8.16	7.00 (±0.50)	6.14–8.95	**<0.001**
	Premolars	3.4	7.03 (±0.50)	5.65–8.30	7.35 (±0.52)	6.22–8.50	**<0.001**
		3.5	7.14 (±0.50)	6.04–8.70	7.37 (±0.58)	6.00–8.98	**0.008**
	Molar	3.6	10.62 (±0.57)	9.10–12.32	11.00 (±0.61)	9.76–12.17	**<0.001**

Note: * Student's *t*-test. Significant *p*-values ($p < 0.05$) denoted in bold.

3.2. Sex Prediction Model Development

In order to develop a model for sex prediction based on the teeth mesiodistal width, a multivariate logistic regression procedure was implemented considering a first stage that included all teeth that exhibited significant differences in the sex-based comparison: canines, premolars and molars. Then, a stepwise adjusted logistic regression procedure derived a reduced best fitting model that is depicted in Table 2. In this final optimized model, the upper left canine (2.3), the lower right lateral incisor (4.2) and the lower right canine (4.3) were the only teeth included.

Table 2. Final reduced logistic regression model (n = 168).

Variables	*B*	*p*-Value	EXP (B)	EXP (B) 95% CI	
				Lower	Upper
Tooth 2.3	1.208	0.019	3.35	1.22	9.22
Tooth 4.3	1.800	0.003	6.05	1.84	19.91
Tooth 4.2	−1.317	0.018	0.27	0.09	0.80
Constant	−14.546	<0.001	-	-	-

R^2(N) = 0.268, % correct classification = 75%; H&L: X^2 = 6.767 (p = 0.562); Note: outcome variable (sex) coded as male: 1, female: 0.

From the fitted model data, the following formula can be derived:

$$\ln(p/(1-p)) = -14.546 + 1.208x + 1.800y - 1.317z$$

where p is the probability of an individual being classified as male, with a lower cutoff value of 0.5, and with x, y, and z representing the mesiodistal width of the upper left canine (2.3), lower right canine (4.3) and lower right lateral incisor (4.2), respectively.

3.3. Comparison with Previous Models

The achieved model was then compared to the previous sex prediction tools by means of performance when applied to this sample (Table 3).

Table 3. Performance assessment of the different models when applied to the studied sample. Measures are presented as percentage. For AUC, estimation by a 95% confidence interval (95% CI) is also shown.

Model		Sensitivity (%)	Specificity (%)	Accuracy (%)	Precision (%)	AUC within ROC Analysis (95% CI)
Acharya et al., 2007 [23]	Model 1	85.3	50.8	73.2	76.2	0.642 (0.551–0.733)
	Model 2	13.8	93.2	41.7	78.9	0.637 (0.546–0.728)
Mitsea et al., 2014 [4]		65.1	45.8	58.3	68.9	0.681 (0.592–0.770)
Peckmann et al., 2016 [5]	Model 1	89.9	39.0	72.0	73.1	0.535 (0.445–0.625)
	Model 2	59.6	49.2	56.0	68.4	0.555 (0.463–0.646)
	Model 3	65.1	45.8	58.3	68.9	0.644 (0.553–0.736)
	Model 4	88.1	37.3	70.2	72.2	0.544 (0.452–0.636)
	Model 5	86.2	37.3	69.0	71.8	0.555 (0.463–0.646)
	Model 6	82.6	45.8	69.6	73.8	0.627 (0.535–0.719)
	Model 7	81.7	45.8	69.0	73.6	0.618 (0.525–0.710)
Neves et al., 2020		87.2	52.5	75.0	77.2	0.768 (0.693–0.843)

AUC—Area under the curve; ROC—Receiver operating characteristic.

4. Discussion

Sex estimation is a vital step in forensic medicine for body identification both in single and mass disaster events. In the Portuguese scenario, sex estimation models through dental hard tissues are scarce and previous proposed models lacked consistency and only accounted for a small subset of

teeth. Therefore, we aimed to develop a potential model using mesiodistal widths of models cast previously for studies of orthodontic indexes. Then, we compared the performance of this model with other full-mouth mesiodistal models published elsewhere. Overall, our model outperformed all available strategies and might be used as a forensic tool for sex estimation in Portuguese samples.

As previously stated, sexual dimorphism may vary between populations, possibly due to a variety of reasons [3,14]. Therefore, this new model arises as a valuable tool to forensic dentistry.

Our results have prospective importance. (1) Until now, there were no models developed for the Portuguese population from complete models. (2) Dental hard tissues are of utmost interest because they are the most lasting tissue of human body, even in post-mortem difficult conditions. (3) This tool may be very useful in single or mass disasters or body identification cases in Portugal, especially due to the unpredictability of these situations. (4) These results confirm sexual dimorphism on teeth mesiodistal width in canines, premolars, and molars of the upper and lower arches.

Dental crown dimensions can be obtained through intraoral measurements [18], dental forms [4,11,13,14,16,19,24], or human remains [20,21]. The mesiodistal and buccolingual measurements of the crown were the two most commonly used and studied dimensions [1,11,14,18–21], followed by diagonal measurements (mesiobuccal-distolingual and distobuccal-mesiolingual) [1,21,25] and the canine mandibular index (expressed as the ratio between the mesiodistal dimension of the canines and the width of the intercanine arch [12,15,19,26]). These studies have shown that canine dimensions provide the highest sexual dimorphism [16,18,21,24,25], followed by premolars [21,25], first and second molars [20,21,25,27].

In this study, we analyzed the degree of sexual dimorphism in different teeth by measuring the maximum mesiodistal diameters of fully erupted permanent teeth from study casts. Overall, several teeth are sexually dimorphic and the crown mesiodistal dimensions were larger on average in males than in females. The results of this study confirm what was previously demonstrated, canine teeth are the most dimorphic teeth [1–5,7,19,21,24–26] but also molars present significant differences between sexes [11,13,23,25,28–30]. Within the elements that fit into our sex prediction model, the upper left canine, the lower right lateral incisor, and the lower right canine were the most appropriate and with better replicability.

Regarding the performance, our developed model outperformed previous published indexes in terms of AUC. In terms of accuracy and precision values, our model also outperformed the remaining models (75.0% and 77.2% for accuracy and precision, respectively). Furthermore, for sensitivity and specificity, this newly developed model presented the best combination of results, only being outperformed in sensitivity by Peckmann et al., 2016 [5] (model 4) and in specificity by Acharya et al., 2007 [23] (model 2).

Like other methodologies used in sex prediction, the amount and quality of evidence available for analysis are critical in forensic investigation. Some limitations of the applied methodology include any post-eruptive changes such as caries, interproximal wear, and interproximal restorations, which compromises the correct measurement of the teeth.

5. Strengths and Limitations

A possible limitation of this study is the fact that we have not accounted for second molars, mainly because the data that this analysis is derived from is an orthodontic population whose main purpose was to study a mesiodistal proportion measure. This measure, Bolton's analysis [31], only accounts for the mesiodistal width from the first molar to the first molar. Nevertheless, two previous large-base studies from this population revealed that second molars are one of the most commonly missing teeth, aside from first molars and premolars [32,33]. Interestingly, none of them accounted for the final model. Another possible limitation is the fact that the new model emerged from the same sample being studied, which may influence the results. The new model should be further investigated with a new sample in a future study.

6. Conclusions

Considering the limitations of this study, the present study found that there is a prevalence of sexual dimorphism in all teeth except the incisors and that the canines exhibit the most noticeable difference between sexes, followed by the first mandibular molars and premolars.

Through a stepwise adjusted logistic regression procedure, a suitable model for sex determination was developed. The reduced model was based on the upper left canine (2.3), the lower right lateral incisor (4.2), and the lower right canine (4.3) and achieved an accuracy of 75%.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/12/4156/s1.

Author Contributions: Conceptualization, V.M. and J.B.; methodology, J.A.N.; validation, J.B., V.M. and L.P.; formal analysis, L.P.; investigation, J.A.N.; resources, A.S.D.; writing—original draft preparation, J.A.N.; writing—review and editing, J.J.M., N.A.-F. and A.Q.; supervision, J.J.M., A.S.D., N.A.-F. and A.Q.; project administration, J.J.M. and A.S.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pereira, C.; Bernardo, M.; Pestana, D.; Santos, J.C.; de Mendonça, M.C. Contribution of teeth in human forensic identification—Discriminant function sexing odontometrical techniques in Portuguese population. *J. Forensic Leg. Med.* **2010**, *17*, 105–110. [CrossRef]
2. Khamis, M.F.; Taylor, J.A.; Malik, S.N.; Townsend, G.C. Odontometric sex variation in Malaysians with application to sex prediction. *Forensic Sci. Int.* **2014**, *234*, 183.e1–183.e7. [CrossRef]
3. Işcan, M.Y.; Kedici, P.S. Sexual variation in bucco-lingual dimensions in Turkish dentition. *Forensic Sci. Int.* **2003**, *137*, 160–164. [CrossRef]
4. Mitsea, A.G.; Moraitis, K.; Leon, G.; Nicopoulou-Karayianni, K.; Spiliopoulou, C. Sex determination by tooth size in a sample of Greek population. *HOMO-J. Comp. Hum. Biol.* **2014**, *65*, 322–329. [CrossRef] [PubMed]
5. Peckmann, T.R.; Logar, C.; Garrido-Varas, C.E.; Meek, S.; Pinto, X.T. Sex determination using the mesio-distal dimension of permanent maxillary incisors and canines in a modern Chilean population. *Sci. Justice* **2016**, *56*, 84–89. [CrossRef] [PubMed]
6. Capitaneanu, C.; Willems, G.; Thevissen, P. A systematic review of odontological sex estimation methods. *J. Forensic Odontostomatol.* **2017**, *35*, 1–19. [PubMed]
7. Isola, G.; Anastasi, G.; Matarese, G.; Williams, R.; Cutroneo, G.; Bracco, P.; Piancino, M. Functional and molecular outcomes of the human masticatory muscles. *Oral Dis.* **2018**, *24*, 1428–1441. [CrossRef]
8. Isola, G.; Alibrandi, A.; Rapisarda, E.; Matarese, G.; Williams, R.C.; Leonardi, R. Association of vitamin D in patients with periodontitis: A cross-sectional study. *J. Periodontal Res.* **2020**, 1–11. [CrossRef]
9. Staderini, E.; Patini, R.; Guglielmi, F.; Camodeca, A.; Gallenzi, P. How to manage impacted third molars: Germectomy or delayed removal? A systematic literature review. *Medicina* **2019**, *55*, 79. [CrossRef]
10. Botelho, J.; Machado, V.; Proença, L.; Delgado, A.S.; Mendes, J.J. Vitamin D deficiency and oral health: A comprehensive review. *Nutrients* **2020**, *12*, 1471. [CrossRef]
11. Angadi, P.V.; Hemani, S.; Prabhu, S.; Acharya, A.B. Analyses of odontometric sexual dimorphism and sex assessment accuracy on a large sample. *J. Forensic Leg. Med.* **2013**, *20*, 673–677. [CrossRef] [PubMed]
12. Silva, A.M.; Pereira, M.L.; Gouveia, S.; Tavares, J.N.; Azevedo, Á.; Caldas, I.M. A new approach to sex estimation using the mandibular canine index. *Med. Sci. Law.* **2016**, *56*, 7–12. [CrossRef] [PubMed]
13. Prabhu, S.; Acharya, A.B. Odontometric sex assessment in Indians. *Forensic Sci. Int.* **2009**, *192*, 129.e1–129.e5. [CrossRef] [PubMed]
14. Brook, A.H.; Griffin, R.C.; Townsend, G.; Levisianos, Y.; Russell, J.; Smith, R.N. Variability and patterning in permanent tooth size of four human ethnic groups. *Arch. Oral Biol.* **2009**, *54*, 79–85. [CrossRef] [PubMed]
15. Rao, N.G.; Rao, N.N.; Pai, M.L.; Kotian, M.S. Mandibular canine index—A clue for establishing sex identity. *Forensic Sci. Int.* **1989**, *42*, 249–254. [CrossRef]

16. Machado, V.; Botelho, J.; Pereira, D.; Vasques, M.; Fernandes-Retto, P.; Proença, L.; Mendes, J.J.; Delgado, A. Bolton ratios in Portuguese subjects among different malocclusion groups. *J. Clin. Exp. Dent.* **2018**, *10*, e864–e868. [CrossRef]
17. Moons, K.G.M.; Altman, D.G.; Reitsma, J.B.; Ioannidis, J.P.A.; Macaskill, P.; Steyerberg, E.W.; Vickers, A.J.; Ransohoff, D.F.; Collins, G.S. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): Explanation and elaboration. *Ann. Intern. Med.* **2015**, *162*, W1–W73. [CrossRef] [PubMed]
18. Khangura, R.; Sircar, K.; Singh, S.; Rastogi, V. Sex determination using mesiodistal dimension of permanent maxillary incisors and canines. *J. Forensic Dent. Sci.* **2012**, *3*, 81. [CrossRef]
19. Phulari, R.S.; Rathore, R.; Talegaon, T.; Jariwala, P. Comparative assessment of maxillary canine index and maxillary first molar dimensions for sex determination in forensic odontology. *J. Forensic Dent. Sci.* **2017**, *9*, 110. [CrossRef]
20. Zorba, E.; Moraitis, K.; Manolis, S.K. Sexual dimorphism in permanent teeth of modern Greeks. *Forensic Sci. Int.* **2011**, *210*, 74–81. [CrossRef]
21. Zorba, E.; Moraitis, K.; Eliopoulos, C.; Spiliopoulou, C. Sex determination in modern Greeks using diagonal measurements of molar teeth. *Forensic Sci. Int.* **2012**, *217*, 19–26. [CrossRef] [PubMed]
22. Glas, A.S.; Lijmer, J.G.; Prins, M.H.; Bonsel, G.J.; Bossuyt, P.M.M. The diagnostic odds ratio: A single indicator of test performance. *J. Clin. Epidemiol.* **2003**, *56*, 1129–1135. [CrossRef]
23. Acharya, A.B.; Mainali, S. Univariate sex dimorphism in the Nepalese dentition and the use of discriminant functions in gender assessment. *Forensic Sci. Int.* **2007**, *173*, 47–56. [CrossRef] [PubMed]
24. Mujib, A.B.R.; Tarigoppula, R.K.V.N.; Kulkarni, P.G. Gender determination using diagonal measurements of maxillary molar and canine teeth in davangere population. *J. Clin. Diagn. Res.* **2014**, *8*, 141–144. [CrossRef]
25. Viciano, J.; Lõpez-Lázaro, S.; Alemán, I. Sex estimation based on deciduous and permanent dentition in a contemporary spanish population. *Am. J. Phys. Anthropol.* **2013**, *152*, 31–43. [CrossRef]
26. Acharya, A.B.; Angadi, P.V.; Prabhu, S.; Nagnur, S. Validity of the mandibular canine index (MCI) in sex prediction: Reassessment in an Indian sample. *Forensic Sci. Int.* **2011**, *204*, 207.e1–207.e4. [CrossRef]
27. Acharya, A.B.; Prabhu, S.; Muddapur, M.V. Odontometric sex assessment from logistic regression analysis. *Int. J. Leg. Med.* **2011**, *125*, 199–204. [CrossRef]
28. Thapar, R.; Angadi, P.V.; Hallikerimath, S.; Kale, A.D. Sex assessment using odontometry and cranial anthropometry: Evaluation in an Indian sample. *Forensic Sci. Med. Pathol.* **2012**, *8*, 94–100. [CrossRef]
29. Filho, I.E.M.; Lopez-Capp, T.T.; Biazevic, M.G.H.; Michel-Crosato, E. Sexual dimorphism using odontometric indexes: Analysis of three statistical techniques. *J. Forensic Leg. Med.* **2016**, *44*, 37–42. [CrossRef]
30. Filipovic, G.; Kanjevac, T.; Cetenovic, B.; Ajdukovic, Z.; Petrovic, N. Sexual dimorphism in the dimensions of teeth in serbian population. *Coll. Antropol.* **2016**, *40*, 23–28. Available online: http://www.ncbi.nlm.nih.gov/pubmed/27301233 (accessed on 7 May 2020).
31. Bolton, W.A. Disharmony in tooth size and its relation to the analysis and treatment of malocclusion. *Angle Orthod.* **1985**, *28*, 113–130.
32. Machado, V.; Botelho, J.; Amaral, A.; Proença, L.; Alves, R.; Rua, J.; Cavacas, M.A.; Delgado, A.S.; Mendes, J.J. Prevalence and extent of chronic periodontitis and its risk factors in a Portuguese subpopulation: A retrospective cross-sectional study and analysis of Clinical Attachment Loss. *PeerJ* **2018**, *6*, e5258. [CrossRef] [PubMed]
33. Botelho, J.; Machado, V.; Proença, L.; Alves, R.; Cavacas, M.A.; Amaro, L.; Mendes, J.J. Study of Periodontal Health in Almada-Seixal (SoPHiAS): A cross-sectional study in the Lisbon Metropolitan Area. *Sci. Rep.* **2019**, *9*, 1–10. [CrossRef] [PubMed]



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3.2. Validation of the Third Molar Maturation Index (I_{3M}) to assess the legal adult age in the Portuguese population

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Validation of the Third Molar Maturation Index (I_{3M}) to assess the legal adult age in the Portuguese population

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Age estimation is a major step in forensic and legal procedures. Its relevance has been increasing due to growing society issues, such as identification of missing people, crimes against minors or lack of valid identification papers from locals or foreigners. Evaluation of the cut-off value of the Third Molar Maturation Index (I_{3M}) = 0.08 for discriminating minors from adults in the Portuguese population. The left lower third molars were analysed by applying a specific cut-off value of 0.08 determined by Cameriere et al. in 2008. A sample of 778 digital panoramic radiographs of a representative Portuguese sample (442 females and 336 males), in the age range of 12–24 years (mean age 17.7 ± 2.98 years in females and 18.1 ± 3.0 years in males), was retrospectively evaluated. I_{3M} decreased as the real age gradually increased in both sexes. The 0.08 cut-off score was valuable in discriminating adults from minors. According to the pooled results, the accuracy, by means of area under the curve, was 92.8% (95% confidence interval (CI) 91.0–94.6%). The proportion of correctly classified subjects (sensitivity) was 90.7% (95% CI 88.7–92.8%) and the specificity was 94.9% (95% CI 93.3–96.4%). The results show that I_{3M} is a valuable method to differentiate minors from adults in the Portuguese population.

Age estimation is a key step in human identification, legal practice and clinical research. Some areas of interest are in the aid of identification of missing people, crimes against minors, adoption procedures, invalid identification documents and mass migration[1–4]. For such reasons, a reliable standardized age estimation tool to differentiate minors from adults is of great interest[1,5].

Based on research on the distinguishable features of skull bones, symphysis pubis, long bones, hand bones and permanent dentition[2,6–10], the Study Group on Forensic Age Diagnostics of the German Society of Legal Medicine (AGFAD) presented the criteria for age estimation in lawsuits. Dental examination is highly reliable because dental development is less influenced by both internal and external factors[1,4]. With the exception of the third molars, permanent dentition completed its development between 12 and 14 years of age. Third molars are estimated to develop between the ages of 15.7 and 23.3 years, and because this timeframe intercrosses the legal age of 18, they can serve as a discriminant tool to distinguish adults from minors[1–3,5].

In Portugal, the age of criminal and legal responsibility is 14 years old. If a crime is committed by an individual between 12 and 16 years old, he will be tried in a juvenile court. In the event of a conviction, the individual may be condemned to serve time in a closed educational center. On the other hand, if the person who commits the crime has between 16 and 18 years of age, may be considered adult and will be judged under general criminal laws. For all purposes, the legal age of adulthood in Portugal is 18 years old[11,12].

In 2008, Cameriere et al.[13] proposed the Third Molar Maturation Index (I_{3M}) as a predictive tool to discriminate adult age. The I_{3M} relies on the relationship between chronological age and the measures of the open apices

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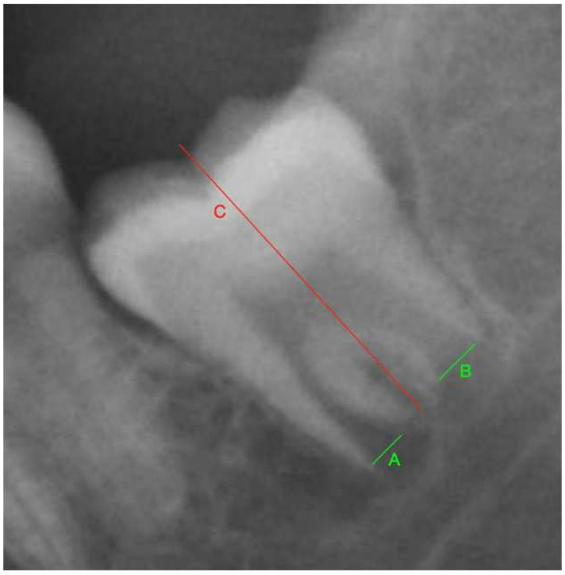


Figure 1. Measurement of the third molar index.

of the lower left third molar. The bidimensional widths of the apical and tooth lengths are measured and used to calculate the I_{3M}. Then, a cutoff value (0.08) is set to differentiate minors from adults[13].

Over the last years, I_{3M} was successfully validated worldwide, in Europe[2,13–23], Africa[1,24–27], Asia[5,28–31], America[3,32–36] and Oceania[37]. Overall, I_{3M} had considerable reliability among all countries, however, to date, this method is not validated for a Portuguese sample. Therefore, investigating if the I_{3M} is suitable for the Portuguese population would be of great interest.

Given the evidence on high population movements in the Portuguese population, contributing to a higher level of diversity than some neighboring populations[38,39], we aimed to test the validity of the I_{3M} in a Portuguese representative sample, by using panoramic radiographs (PRs).. Secondly, we looked for the influence of sex on its validity. Our null hypothesis was that the I_{3M} has no validity to discriminate adult age in this Portuguese sample.

Materials and methods

Source of data and sample size. This retrospective observational study has received approval from the Egas Moniz Ethics Committee (ID 887). Written informed consent was obtained for each participant, during the first appointment at the Egas Moniz Dental Clinic (EMDC), (Almada, Portugal). Regarding participants under 18, a parent and/or legal guardian and this study gave and signed the informed consent. This research was conducted in accordance with the Declaration of Helsinki, as revised in 2013.

The present study follows the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) reporting guidelines[40] for validation of prediction models. This study was conducted on a triple-blind basis with respect to: (1) diagnosis and clinical outcome; (2) data collection; and (3) analysis.

Participants. A consecutive sample of 1267 digital PRs, taken between September 2017 and January 2020, were considered for this study.

The inclusion criteria were: the presence of the lower third molar; known chronological age between 12 and 24 years old[5,13,36]; and absence of evident bone pathologies or systemic diseases that may affect tooth development. Exclusion criteria included spatial orientation of the third molar that prevents correct measuring, endodontic treatment and/or coronary restoration on the third molar, extensive cavities or abnormal dental anatomy, congenital anomalies and poor-quality or distorted X-rays[41].

Outcome, predictors and measurement reproducibility. All digital PRs were converted to JPEG documents for the examination with ImageJ image processing software (Graphics Suite X7, Ottawa, Canada) by one trained and calibrated observer (JAN). The I_{3M} index of each evaluated third molar was performed according to the Cameriere et al. method[13]. If the root development of the third molar is complete, then $I_{3M} = 0.0$. If not, I_{3M} was calculated as the distance between the inner sides of the open apex (A and B) divided by the tooth length (C) (Fig. 1). In case of $I_{3M} < 0.08$, the individual is classified as 18 years old or older and if $I_{3M} \geq 0.08$, the individual as considered a minor.

The Kappa correlation index was used to test the agreement of classification of individuals younger than 18 years, aged 18 years or older. The Intraclass Correlation Coefficient (ICC) was used to analyze the measurements of open apices. Previously, twelve PRs were randomly chosen from the total sample, measured and

Sensitivity	TP/(TP + FN)	Proportion positive test results among diseased
Specificity	TN/(TN + FP)	Proportion negative test results among the "healthy"
Accuracy	(TP + TN)/(TP + TN + FP + FN)	Proportion of correctly identified subjects
PPV	TP/(TP + FP)	-
LR +	Sensitivity/(1 – Specifity)	Ratio of the probability a true negative is classified as a true negative
LR-	(1 – Sensitivity)/Specificity	Ratio of the probability a false negative is classified as a true negative
Youden's index	Sensitivity + Specificity – 1	Measures the performance of a dichotomous diagnostic test
F1 Score	2TP/(2TP + FP + FN)	Harmonic mean of precision and sensitivity
MCC	(TP × TN – FP × FN)/SQRT[(TP + FP)(TP + FN)(TN + FP)(TN + FN)]	Measure of quality of binary classifications

Table 1. Diagnostic performance indicators used in the comparative analysis. *FN* False Negative, *FP* False Positive; *TN* True Negative; *TP* True Positive; *PPV* Positive Predictive Values; *MCC* Matthews Correlation Coefficient. Adapted from Glas et al. (2003).

remeasured one week later by the same researcher (JAN). Kappa index indicated a perfect agreement for the inter-examiner analysis (κ = 1.00). Intraclass correlation coefficient (ICC) showed absolute agreement (ICC = 0.96), according to Landis and Koch[42].

Statistical analysis. Data analysis was performed using SPSS Statistics v. 25.0 for Windows (IBM; Armonk, New York, USA). The estimated I_{3M} index result was compared against the actual chronological age to determine the method's performance in global and according to sex (females and males). For this purpose, contingency tables were used to calculate true positive (TP), true negative (TN), false positive (FP) and false negative (FN) values (Table 1). Then, several performance indicators were determined and detailed in Table 1[43]. Performance measurement was assessed through binary and multiclass area under the curve (AUC), through receiver operating characteristics (ROC) analysis. The correspondent 95% confidence intervals (95% CI) were also determined. Bayes post-test probability (p) of being 18 years or older is computed to discriminate between those who are or are not aged 18 years or more. According to Bayes' theorem, p may be written as:

$$p = \frac{Se\,x\,po}{Se\,x\,po + (1 - Sp)(1 - po)}$$

In the post-test probability p, $p0$ defines the probability that a participant is 18 years or older given that he or she is aged between 12 and 24 years, in the target population. In this study, we calculated the probability $p0$ as the proportion of participants between 18 and 24 years of age who live in Portugal and those who are aged between 12 and 24 years. This value ($p0$) was considered to be 0.55 (global, males and females) according to the Portuguese National Statistics Institute - Instituto Nacional de Estatística (INE) (https://www.ine.pt/xportal).

Results

Participants. A total of 778 digital PRs (336 males and 442 females) met the inclusion criteria, with 489 being excluded due to absence of third molars. The distribution of age and sex is depicted in Table 2. The mean ages of the males and females, aged between 12 and 24 years, were 18.1 ± 3.0 years old and 17.7 ± 3.0, respectively, without statistically significant difference ($p = 0.078$).

Model performance. The estimated age of majority was correlated with the chronological age ($p < 0.001$). The I_{3M} values decreased as age increased across all age groups in both sexes, showing that the lower third molar mineralization occurred earlier in females than in males (Fig. 2).

Then, pooled data of both sexes as well as separately was analysed (Table 3). The overall results were 92.8% (95% CI 91–94.6%), 90.7% (95% CI 88.7–92.8%) and 94.9% (95% CI 93.3–96.4%) for accuracy, sensitivity and specificity, respectively. Concerning the LR+ and the LR−, their values were as follows: 17.7 (95% CI 12.5–20.3) and 0.10 (95% CI 0.08–0.12). Global post-test probability was 95.6% (95% CI 94.1–97.0%).

Males were better classified into adults or minors (94%; 95% CI 92.4–95.7%) than females (91.2%; 95% CI 89.9–93.8%). Specificity was better for females (95.7%; 95% CI 94.3–97.1%) compared to males (93.5%; 95% CI 91.8–95.3%) while sensitivity was better for males (94.9%; 95% CI 93.3–96.4%) compared to females (87.5%; 95% CI 85.2–89.8%). Both estimating post-test probability results were excellent; in males was 94.7% (95% CI 93.1–96.3%) and 96.2% (95% CI 94.2–97.5%) in females. The LR+ and LR− were 20.5 (95% CI 15.0–23.3) and 0.13 (95% CI 0.11–0.15), respectively, in females and 14.6 (95% CI 9.9–17.1) and 0.06 (95% CI 0.04–0.08), respectively, in males.

Data from articles showing results with cutoff value 0.08 in various European samples. Also, we compared the results of the present study with previous European studies (Tables 4 and 5 present).

Age (years)	Males	Females	Total
12	13	16	29
13	11	22	33
14	10	21	31
15	29	36	65
16	41	64	105
17	51	75	126
18	36	43	79
19	43	38	81
20	28	44	72
21	15	28	43
22	20	22	42
23	27	16	43
24	12	17	29
Total	336	442	778

Table 2. Participants age and sex distribution (n = 778).

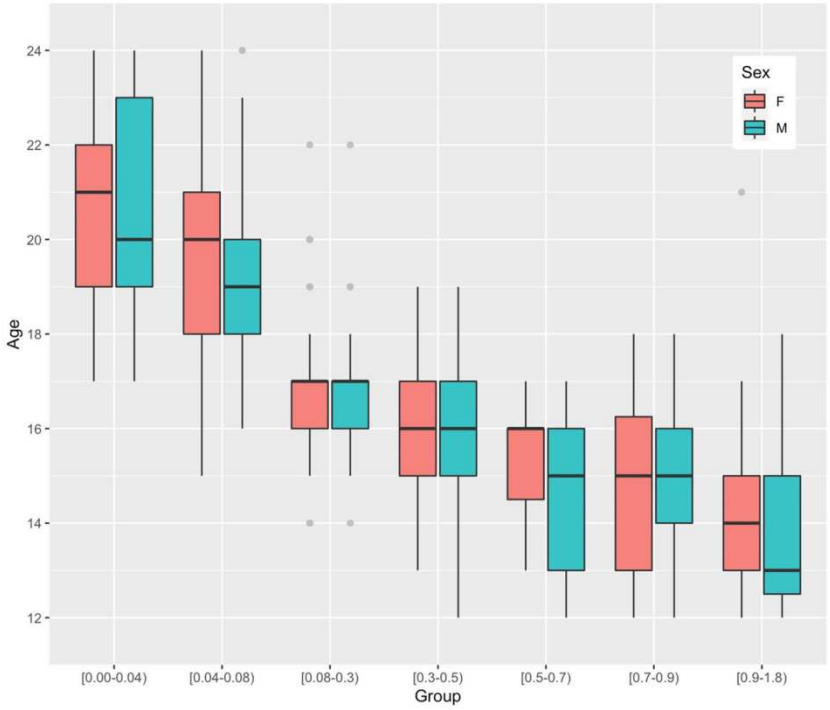


Figure 2. Boxplot of relationship between chronological age and I_{3M} of open apices of lower left third molar, according to females and males.

Discussion

The present study is the first to test the validity of I_{3M} in a Portuguese sample. Overall, the null hypothesis was rejected, that is, the I_{3M} is a reliable tool to discriminate adult age in this Portuguese population. Further, I_{3M} was more accurate in male participants. These results have important implications because this tool has potential to be used in legal and criminal settings.

Discriminating minors from adults is important to prevent legal wrongful procedures, specially in cases where valid identification documents are lacking, in order to prevent legal wrongful procedures[4,32]. Notwithstanding, age estimation is challenging, particularly in the differentiation teens from young adults (aged 15 years old to early 20 s), as the physical appearance and characterization are not clearly related of being an adult[1,4]. From 15 years of age, the third molars are only teeth not fully developed, as this clinical circumstance is useful in forensic sciences[44].

	Global	Female	Male
Sensitivity	90.7 (88.7–92.8)	87.5 (85.2–89.8)	94.5 (92.9–96.1)
Specificity	94.9 (93.3–96.4)	95.7 (94.3–97.1)	93.5 (91.8–95.3)
Accuracy	92.8 (91.0–94.6)	91.9 (89.9–93.8)	94.0 (92.4–95.7)
Precision	94.6 (93.1–96.2)	94.8 (93.2–96.4)	94.5 (92.9–96.1)
LR+	17.7 (12.5–20.3)	20.5 (15.0–23.3)	14.6 (9.9–17.1)
LR−	0.10 (0.08–0.12)	0.13 (0.11–0.15)	0.06 (0.04–0.08)
AUC	92.8 (90.7–94.9)	91.6 (88.6–94.6)	94.0 (91.1–97.0)
Bayes *p*	95.6 (94.1–97.0)	96.2 (94.8–97.5)	94.7 (93.1–96.3)
Youden's index	85.6 (83.1–88.1)	83.2 (80.6–85.9)	88.0 (85.7–90.3)
F1 Score	92.7 (90.8–94.5)	91.0 (89.0–93.0)	94.5 (92.9–96.1)
MCC	85.7 (83.2–88.1)	83.8 (81.2–86.4)	88.0 (85.7–90.3)

Table 3. Performance values (in percentage), derived from 2 × 2 contingency tables (with correspondent 95% confidence intervals), of test of age of majority in the Portuguese population.

Author	Country/Region	n	Accuracy	Sensitivity	Specificity	Post-test probability
Cameriere et al. (2008)*	Macerata, Italy	906	83.0 (80.6–84.5)	70.0 (67.0–73.0)	98.0 (97.1–98.9)	98.0 (97.0–99.0)
De Luca et al. (2014)*	Milan, Italy	397	91.4 (82.8–99.9)	86.6 (88.8–91.1)	95.7 (92.1–98.0)	95.6 (92.0–98.0)
Cameriere et al. (2014)*	Rome, Italy	287	88.5 (84.8–92.2)	84.1 (76.7–89.9)	92.5 (87.0–96.2)	90.1 (83.6–95.2)
Rozyło-Kalinowska*et al.* (2017)	Poland	982	86.6 (84.4–88.7)	84.6 (81.9–87.2)	92.0 (88.7–95.3)	96.6 (93.6–99.6)
Spinas et al. (2018)	Sardinia, Italy	336	86.0 (82.0–89.0)	82.0 (76.0–86.0)	95.0 (89.0–97.0)	–

Table 4. Global performance values (in percentage, with correspondent 95% confidence intervals), of test of age of majority in several European samples. *In studies reporting validation of I_{3M} method not reporting 95% Confidence Interval (CI) we calculated 95% CI following Higgins et al. (2011).

Author	Country/ Region	n	Accuracy		Sensitivity		Specificity		Post-test probability	
			Male	Female	Male	Female	Male	Female	Male	Female
Galic et al. (2014)	Croatia	1416	91.5 (89–93.5)	88.8 (86.3–90.9)	91.2 (88.7–93.1)	84.3 (80.6–87.5	91.9 (88.8–94.3)	95.4 (92.5–97.5)	94.5 (94.3–94.7)	96.5 (95.9–97)
Cameriere et al. (2014)	Albania	298	92.5 (89.9–96.2)	87.5 (81.2–90.4)	94.1 (87.6–97.8)	75.4 (68.1–78.8)	90.9 (84.2–94.7)	96.6 (91.1–99.1)	94.4 (88.7–97.3)	97.2 (91.9–99.1)
Zelic et al. (2016)	Serbia	589	95.0 (92.0–98.0)	91.0 (87.0–92.0)	96.0 (93.0–98.0)	86.0 (83.0–87.0)	94.0 (90.0–98.0)	98.0 (94.0–99.0)	96.0 (91.0–100)	99.0 (93.0–100)
Gulsahi et al. (2016)*	Turkey	293	97.6 (94.9–100)	92.7 (88.7–96.6)	94.6 (88.1–99.8)	85.9 (77.1–92.8)	100	100	100	100
Rozyło-Kalinowska et al. (2017)	Poland	982	87.6 (84.8–90.3)	85.3 (82–88.6)	86.2 (82.8–89.6)	82.6 (78.4–86.7)	91.2 (86.7–95.8)	93 (88.3–97.7)	96.3 (92.3–100)	97.0 (92.4–100)
Kelmendi et al. (2017)	Kosovo	1221	96.8 (92.6–98.5)	90.9 (87–91.7)	96.2 (92.5–97.8)	82.6 (78.7–83.4)	97.6 (92.9–99.5)	99.1 (95.3–100)	97.5 (90.5–100)	98.9 (92.6–100)
Dogru et al. (2017)	Netherlands	360	88.9 (83.3–91.8)	83.3 (77.7–85.8)	84.0 (78.9–86.6)	72.7 (67.6–75)	95.0 (88.7–98.3)	96.3 (90.0–99.0)	95.7 (88.4–100)	96.3 (89–1-100)
Spinas et al. (2018)	Sardinia, Italy	336	87.0 (82.0–91.0)	84.0 (78.0–89.0)	85.0 (78.0–91.0)	79.0 (71.0–85.0)	91.0 (82.0–96.0)	100 (92.0–100)	-	-
Tafrount et al. (2018)	France	339	91.6 (87.1–90.8)	89.7 (84.2–92.5)	87.1 (80.4–83.4)	81.3 (75–84.5)	95.3 (89.8–98.3)	96.2 (91.3–97)	95.5 (87.7–100)	96.1 (89.1–100)
Antunović et al. (2018)	Montenegro	683	93.0 (90.0–96.0)	89.0 (85.0–91.0)	92.0 (88.0–96.0)	82.0 (79.0–94.0)	94.0 (90.0–98.0)	96.0 (93.0–98.0)	96.0 (90.0–100)	97.0 (92.0–100)

Table 5. Performance values (in percentage, with correspondent 95% confidence intervals), discriminated by sex, of test of age of majority in several European samples. *In studies reporting validation of I_{3M} method not reporting 95% Confidence Interval (CI) we calculated 95% CI following Higgins et al. (2011).

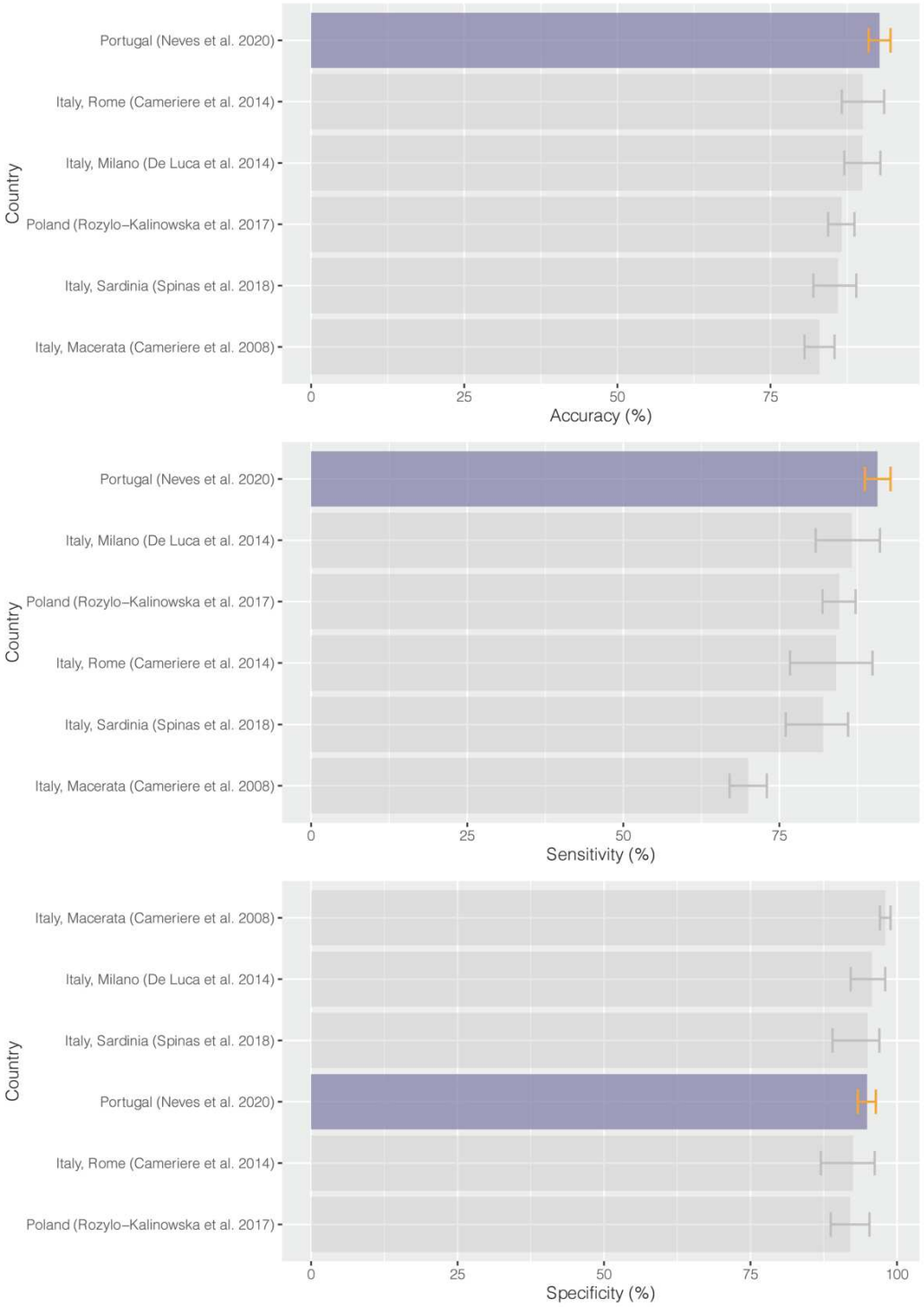


Figure 3. Pooled results from different European populations.

In the Portuguese context, age estimation methods involving dental measures were reported in previous studies[45–50]. Caldas et al. (2011)[46] have evaluated third molars, however the method and the age range of these

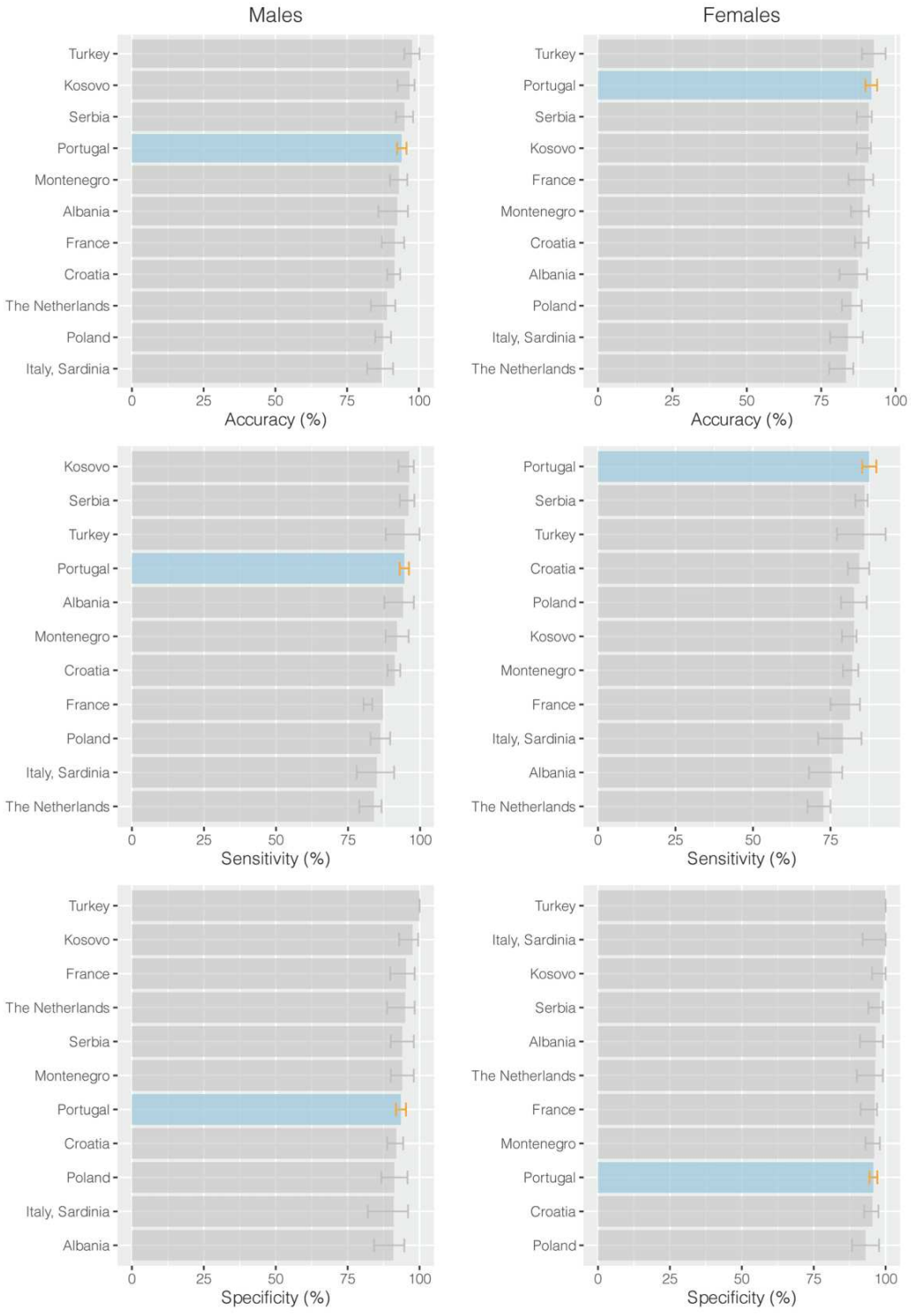


Figure 4. Discriminated results by sex, from different European populations.

participants were not comparable. Thus, to the best of our knowledge, this is the first study that validates the use of the I_{3M} cutoff value of 0.08 in the Portuguese population.

Cameriere et al. (2008)[13] introduced a new methodology for age estimation based on the ratio between measures of the open apices and height of the third molar. Since then, the I_{3M} has been validated in every continent with very strong results, showing only slight variations among different ethnicities.

The sensibility indicates the I_{3M} ability to correctly identify individuals who are 18 years or older ($I_{3M} < 0.08$). On the other hand, specificity is the ability to discriminate individuals younger than 18 years old ($I_{3M} \geq 0.08$). Santiago et al. (2018)[41] reported that I_{3M} outperforms in individuals younger than 18 years due to the higher specificity of this tool. This is key in forensic science because if a minor is wrongly processed as an adult it would violate its rights[23,41].

To better integrate the results of our study within the European scenario, we analyzed every European study that have analyzed the validity of I_{3M} (Fig. 3). Our pooled overall results on accuracy (92.8%; 95% CI 91.0–94.6%) outperformed all previous studies that presented pooled data[2,13,17,22,23] . Both sensitivity and specificity pooled results also surpassed most previous European studies[2,22,23] except for Cameriere et al.[13] (98%) and De Luca et al.[17] (95.7%; 95% CI 92.1–98.0%). The estimated post-test probability of the pooled data (95.6%; 95% CI 94.1–97.0%) showed similar results with previous studies.

When analysing our data regarding males and females (Fig. 4), we demonstrated alike maturation of third molars in both males and females, which is in line with similar studies[14–16,19–23,44,51]. Previous European studies present slightly better accuracy and sensibility in males than in females[14–16,19–23,44,51]. Contrary, females presented better specificity than males[14–16,19–23,44,51]. Therefore, I_{3M} seems to be slightly more accurate in males, although it is equivalent to the female participants in this Portuguese population. The effect of age on I_{3M} performance is proposed to rely on the maturation development according to sex as women tend to develop at a younger ages than men[52]. This maturation difference is suggested to explain the I_{3M} results discrepancies between both sexes, however more studies are needed to fully understand the biological reasons upon this subject[19,41,51].

Estimated post-test probability of both male (94.7%; 95% CI 93.1–96.3%) and female (96.2%; 95% CI 94.8–97.5%) demonstrated results compatible with other European studies[2,14,15,19–23,44,51]. It is important to refer that Zelic et al.[20] and Kelmendi et al.[21] reported results very close to 100% in females, and Gulsahi et al.[19] reported results of 100% in both sexes, although the sample size was one of the smallest in all European studies (n = 293), which may influence these results.

The likelihood ratio is a practical measure of diagnostic accuracy. The higher the value of LR + , more the test has the capability of establishing the tested condition; an LR + value greater than 10 defines it as a good diagnostic test. In our study, balanced values of LR + and LR – have been achieved. Although I_{3M} presented higher LR + in females (20.5; 95% CI 15–23.3) then males (14.6; 95% CI 9.9–17.1), I_{3M} is an excellent prediction of the probability of majority; and lower LR – (0.06; 95% CI 0.04–0.08) in males than (0.13; 95% CI 0.11–0.15) in females, prove that the test is also an excellent tool at classifying individuals younger than 18 years of age, despite the sex of the individuals[19,23].

In conclusion, I_{3M} is a suitable method in legal and forensic purposes to identify minors from adults in the Portuguese population. Further, the cut-off value used (0.08) is predictable and useful to discriminating individuals younger than 18 years of age (high specificity in both sexes).

Received: 11 July 2020; Accepted: 13 October 2020
Published online: 28 October 2020

References

1. Angelakopoulos, N. *et al.* Third molar maturity index (I 3M) for assessing age of majority: study of a black South African sample. *Int. J. Legal Med.* **132**, 1457–1464 (2018).
2. Cameriere, R. *et al.* Assessment of legal adult age of 18 by measurement of open apices of the third molars: Study on the Albanian sample. *Forensic Sci. Int.* **245**(205), e1-205.e5 (2014).
3. da Nóbrega, J. B. M. *et al.* Validation of the Third Molar Maturation Index to estimate the age of criminal responsibility in Northeastern Brazil. *Forensic Sci. Int.* **304**, 109917 (2019).
4. Cunha, E. *et al.* The problem of aging human remains and living individuals: a review. *Forensic Sci. Int.* **193**, 1–13 (2009).
5. Chu, G. *et al.* Third molar maturity index (I3M) for assessing age of majority in northern Chinese population. *Int. J. Legal Med.* **132**, 1759–1768 (2018).
6. Ozveren, N., Serindere, G., Meric, P. & Cameriere, R. A comparison of the accuracy of Willems' and Cameriere's methods based on panoramic radiography. *Forensic Sci. Int.* **302**, 109912 (2019).
7. Cameriere, R., Cingolani, M., Giuliodori, A., De Luca, S. & Ferrante, L. Radiographic analysis of epiphyseal fusion at knee joint to assess likelihood of having attained 18 years of age. *Int. J. Legal Med.* **126**, 889–899 (2012).
8. Liversidge, H. M., Speechly, T. & Hector, M. P. Dental maturation in British children: are Demirjian's standards applicable?. *Int. J. Paediatr. Dent.* **9**, 263–269 (1999).
9. Garamendi, P. M., Landa, M. I., Ballesteros, J. & Solano, M. A. Reliability of the methods applied to assess age minority in living subjects around 18 years old: a survey on a Moroccan origin population. *Forensic Sci. Int.* **154**, 3–12 (2005).
10. Garamendi, P. M., Landa, M. I., Botella, M. C. & Alemán, I. Forensic age estimation on digital X-ray images: medial epiphyses of the clavicle and first rib ossification in relation to chronological age. *J. Forensic Sci.* **56**, 3–12 (2011).
11. Cipriani, D. Children's rights and the minimum age of criminal responsibility: a global perspective. *Br. J. Criminol.* **50**, 990–991 (2010).
12. Justiça, M. da. Diário da República nº273. Decreto de Lei nº496/77 (1977).
13. Cameriere, R., Ferrante, L., De Angelis, D., Scarpino, F. & Galli, F. The comparison between measurement of open apices of third molars and Demirjian stages to test chronological age of over 18 year olds in living subjects. *Int. J. Legal Med.* **122**, 493–497 (2008).
14. Tafrount, C., Galić, I., Franchi, A., Fanton, L. & Cameriere, R. Third molar maturity index for indicating the legal adult age in southeastern France. *Forensic Sci. Int.* **294**(218), e1-218.e6 (2019).
15. Boyacıoğlu Doğru, H. *et al.* Age of majority assessment in Dutch individuals based on Cameriere's third molar maturity index. *Forensic Sci. Int.* **282**, 231.e1-231.e6 (2018).
16. Cameriere, R. *et al.* Adult or not? Accuracy of Cameriere's cut-off value for third molar in assessing 18 years of age for legal purposes. *Minerva Stomatol.* **63**, 283–294 (2014).
17. De Luca, S. *et al.* Accuracy of Cameriere's cut-off value for third molar in assessing 18 years of age. *Forensic Sci. Int.* **235**(102), e1-102.e6 (2014).
18. Galić, I. *et al.* Cameriere's third molar maturity index in assessing age of majority. *Forensic Sci. Int.* **252**, 191 e1-191.e5 (2015).

19. Gulsahi, A., De Luca, S., Cehreli, S. B., Tirali, R. E. & Cameriere, R. Accuracy of the third molar index for assessing the legal majority of 18 years in Turkish population. *Forensic Sci. Int.* **266**, 584 e1-584.e6 (2016).
20. Zelic, K. *et al.* Accuracy of Cameriere's third molar maturity index in assessing legal adulthood on Serbian population. *Forensic Sci. Int.* **259**, 127–132 (2016).
21. Kelmendi, J. *et al.* The third molar maturity index in indicating the legal adult age in Kosovar population. *Int. J. Legal Med.* **132**, 1151–1159 (2018).
22. Różyło-Kalinowska, I., Kalinowski, P., Kozek, M., Galić, I. & Cameriere, R. Validity of the third molar maturity index I3M for indicating the adult age in the Polish population. *Forensic Sci. Int.* **290**(352), e1-352.e6 (2018).
23. Spinas, E., De Luca, S., Lampis, L., Velandia Palacio, L. A. & Cameriere, R. Is the third molar maturity index (I3M) useful for a genetic isolate population? Study of a Sardinian sample of children and young adults. *Int. J. Legal Med.* **132**, 1787–1794 (2018).
24. Cavrić, J. *et al.* Third molar maturity index (I3M) for assessing age of majority in a black African population in Botswana. *Int. J. Legal Med.* **130**, 1109–1120 (2016).
25. Dardouri, A. A. K., Cameriere, R., De Luca, S. & Vanin, S. Third molar maturity index by measurements of open apices in a Libyan sample of living subjects. *Forensic Sci. Int.* **267**, 230 e1-230.e6 (2016).
26. AlQahtani, S., Kawthar, A., AlAraik, A. & AlShalan, A. Third molar cut-off value in assessing the legal age of 18 in Saudi population. *Forensic Sci. Int.* **272**, 64–67 (2017).
27. El-Bakary, A. A., El-Azab, S. M., Abou El Atta, H. M., Palacio, L. A. V. & Cameriere, R. Accuracy of the cutoff value of the third molar maturity index: an Egyptian study. *Egypt. J. Forensic Sci.* **9**, 52 (2019).
28. Balla, S. B. *et al.* Validation of third molar maturity index (I3M) for discrimination of juvenile/adult status in South Indian population. *J. Forensic Leg. Med.* **49**, 2–7 (2017).
29. Sharma, P., Wadhwan, V., Ravi Prakash, S., Aggarwal, P. & Sharma, N. Assessment of age of majority by measurement of open apices of the third molars using Cameriere's third molar maturity index. *J. Forensic Dent. Sci.* **9**, 96 (2017).
30. Kumagai, A. *et al.* Accuracy of the third molar index cut-off value for estimating 18 years of age: validation in a Japanese samples. *Leg. Med.* **38**, 5–9 (2019).
31. Jiménez, L. G. *et al.* Validation of the third molar maturity index (I3M): study of a Dominican Republic sample. *J. Forensic Odontostomatol.* **37**, 27–33 (2019).
32. Deitos, A. R. *et al.* Age estimation among Brazilians: Younger or older than 18?. *J. Forensic Leg. Med.* **33**, 111–115 (2015).
33. De Luca, S. *et al.* Accuracy of cut-off value by measurement of third molar index: study of a Colombian sample. *Forensic Sci. Int.* **261**(160), e1-160.e5 (2016).
34. Quispe Lizarbe, R. J., Solís Adrianzén, C., Quezada-Márquez, M. M., Galić, I. & Cameriere, R. Demirjian's stages and Cameriere's third molar maturity index to estimate legal adult age in Peruvian population. *Leg. Med.* **25**, 59–65 (2017).
35. Khare, P. *et al.* Validation of the third molar maturity index cut-off value of <0.08 for indicating legal age of 18 years in Eastern Chinese region. *Leg. Med.* **42**, 101645 (2020).
36. Cameriere, R. *et al.* Assessment of second (I2M) and third (I3M) molar indices for establishing 14 and 16 legal ages and validation of the Cameriere's I3M cut-off for 18 years old in Chilean population. *Forensic Sci. Int.* **285**(205), e1-205.e5 (2018).
37. Franklin, D. *et al.* Accuracy of a cut-off value based on the third molar index: validation in an Australian population. *Forensic Sci. Int.* **266**(575), e1-575.e6 (2016).
38. Pereira, L., Prata, M. J. & Amorim, A. Diversity of mtDNA lineages in Portugal: Not a genetic edge of European variation. *Ann. Hum. Genet.* **64**, 491–506 (2000).
39. Pimenta, J. *et al.* Spatially explicit analysis reveals complex human genetic gradients in the Iberian Peninsula. *Sci. Rep.* **9**, 1–9 (2019).
40. Collins, G. S. *et al.* Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): explanation and elaboration. *Ann. Intern. Med.* **162**, W1–W73 (2015).
41. Santiago, B. M., Almeida, L., Cavalcanti, Y. W., Magno, M. B. & Maia, L. C. Accuracy of the third molar maturity index in assessing the legal age of 18 years: a systematic review and meta-analysis. *Int. J. Legal Med.* **132**, 1167–1184 (2018).
42. Landis, J. R. & Koch, G. G. The measurement of observer agreement for categorical data. *Biometrics* **33**, 159 (1977).
43. Glas, A. S., Lijmer, J. G., Prins, M. H., Bonsel, G. J. & Bossuyt, P. M. M. The diagnostic odds ratio: a single indicator of test performance. *J. Clin. Epidemiol.* **56**, 1129–1135 (2003).
44. Galić, I. *et al.* Dental age estimation on Bosnian–Herzegovinian children aged 6–14 years: evaluation of Chaillet's international maturity standards. *J. Forensic Leg. Med.* **20**, 40–45 (2013).
45. Tomás, L. F., Mónico, L. S. M., Tomás, I., Varela-Patiño, P. & Martin-Biedma, B. The accuracy of estimating chronological age from Demirjian and Nolla methods in a Portuguese and Spanish sample. *BMC Oral Health* **14**, 1–12 (2014).
46. Caldas, I. M. *et al.* Chronological age estimation based on third molar development in a Portuguese population. *Int. J. Legal Med.* **125**, 235–243 (2011).
47. Cameriere, R., Cunha, E., Sassaroli, E., Nuzzolese, E. & Ferrante, L. Age estimation by pulp/tooth area ratio in canines: study of a Portuguese sample to test Cameriere's method. *Forensic Sci. Int.* **193**(128), e1-128.e6 (2009).
48. Anastácio, A. C., Serras, C., Vargas Sousa de Santos, R. F. & Palmela Pereira, C. Validation of Cameriere's medical-legal age estimation method using seconds premolars in a Portuguese population. *J. Forensic Leg. Med.* **60**, 30–34 (2018).
49. Pavlović, S., Palmela Pereira, C. & Vargas Sousa de Santos, R. F. Age estimation in Portuguese population: the application of the London atlas of tooth development and eruption. *Forensic Sci. Int.* **272**, 97–103 (2017).
50. Pereira, C. P., Caldas, R. & Pestana, D. Legal medical age estimation in Portuguese adult cadavers: evaluation of the accuracy of forensic dental invasive and non-invasive methods. *J. Forensic Sci. Criminol.* **1**, 1–6 (2013).
51. Antunovic, M. *et al.* The third molars for indicating legal adult age in Montenegro. *Leg. Med.* **33**, 55–61 (2018).
52. Veldhuis, J. D., Roemmich, J. N. & Rogol, A. D. Gender and sexual maturation-dependent contrasts in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females: a general clinical research center-based study. *J. Clin. Endocrinol. Metab.* **85**, 2385–2394 (2000).

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Conceptualization, V.M, J.B and R.C.; methodology, J.N.; validation, J.B., V.M. and L.P.; formal analysis, L.P.; investigation, J.N.; resources, A.D. and J.J.M.; writing—original draft preparation, J.N.; writing—review and editing, J.J.M., N.A.F., A.Q. and R.C.; supervision, J.J.M, A.D, N.A.F and A.Q.; project administration, J.J.M, A.D. and R.C. . All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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CHAPTER 4

4. Level And Quality Of Previously Published Literature Regarding The Determination And Prediction Of Sex And Age In Forensic Dentistry

This chapter was based on the published work:

Paper III – João Albernaz Neves, Nathalie Antunes-Ferreira, Vanessa Machado, João Botelho, Luís Proença, Alexandre Quintas, Ana Sintra Delgado, José João Mendes. An Umbrella Review of the Evidence of Sex Determination Procedures in Forensic Dentistry. J. Pers. Med. **2022**, 12, 787. 10.3390/jpm12050787

Paper IV – João Albernaz Neves, Luísa Bandeira Lopes, Vanessa Machado, João Botelho, Ana Sintra Delgado, José João Mendes. Evidence of Age Estimation Procedures in Forensic Dentistry: Results from an Umbrella Review. *Preprints* **2023**, 2023101771. https://doi.org/10.20944/preprints202310.1771.v1 (under review)

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4.1. An Umbrella Review of the evidence of sex determination procedures in Forensic Dentistry

Journal of *Personalized Medicine*

MDPI

Review

An Umbrella Review of the Evidence of Sex Determination Procedures in Forensic Dentistry

João Albernaz Neves [1,*], Nathalie Antunes-Ferreira [2], Vanessa Machado [1,3], João Botelho [1], Luís Proença [4], Alexandre Quintas [2], Ana Sintra Delgado [1,3] and José João Mendes [1]

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Abstract: Sex determination in forensic dentistry is a major step towards postmortem profiling. The most widely recognized method is DNA, yet its application in the dental field of forensic sciences is still impractical. Depending on the conditions of the remains, teeth are often the only surviving organ. Some systematic reviews (SRs) have been recently produced; hence this umbrella review critically assesses their level of evidence and provides an overall comprehensive view. An electronic database search was conducted in four databases (PubMed, Cochrane, Web of Science, and LILACS) and three grey search engines up to December 2021, focusing on SRs of sex determination through forensic dentistry procedures. The methodological quality of the SRs was analyzed using the measurement tool to assess SRs criteria (AMSTAR2). Five SRs were included, two of critically low quality and three of low quality. The SRs posited that canines are the most dimorphic teeth; oral tissue remnants are a rich source for sex determination by DNA tracing; and artificial intelligence tools demonstrate high potential in forensic dentistry. The quality of evidence on sex determination using dental approaches was rated as low. Well-designed clinical trials and high standard systematic reviews are essential to corroborate the accuracy of the different procedures of sex determination in forensic dentistry.

Keywords: forensic dentistry; sex determination; sexual dimorphism; dental measurements; predictive models

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Citation: Albernaz Neves, J.; Antunes-Ferreira, N.; Machado, V.; Botelho, J.; Proença, L.; Quintas, A.; Sintra Delgado, A.; Mendes, J.J. An Umbrella Review of the Evidence of Sex Determination Procedures in Forensic Dentistry. *J. Pers. Med.* **2022**, *12*, 787. https://doi.org/10.3390/jpm12050787

Academic Editors: Aristotelis Chatziioannou and Yudong Zhang

Received: 17 April 2022
Accepted: 10 May 2022
Published: 13 May 2022

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1. Introduction

Teeth are highly resistant to aggressive agents, each tooth piece morphology is peculiar, radiographic recording is routinely conducted in standard dental treatment, and the methodological analysis is technically easy and affordable [1–3].

Precise sex prediction is a key step for a postmortem forensic profile [4]. In standard dental measures, teeth have a high degree of sexual dimorphism [5–8]. Usually, male teeth are bigger than female teeth; nevertheless, data is not consensual and reverse dimorphism also takes place [6,9]. Sexual dimorphism may alter between different populations, due to the environment, available food resources, or genetic pool [9,10].

Forensic identification of an individual's remains can be executed using dental methods, such as the palatal rugae [11], the development stage of third molars [12], odontological measures [2,4,6], or alternative sources of DNA [13]. Given the applicability of these strategies, dental-based forensic methods have been gaining some importance in forensic identification.

In comparison with other nonmetric and metric measurements, DNA analysis has greater accuracy in sex determination and was consequently the standard method [14]. Therefore, odontometric measures are only truly valid and useful when sex organs or other sexual characteristics are not present [5].

As a result, this umbrella review intended to evaluate the existing evidence on sex determination procedures in forensic dentistry. Our focus was two-fold: to ascertain its quality of evidence and the overall clinical accuracy of each procedure.

2. Materials and Methods

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed [15] (Supplementary Table S1) as well as the guide for systematic reviews of systematic review [16]. The research question was defined: "What is the current evidence on sex determination approaches in forensic dentistry?".

2.1. Eligibility Criteria

To answer the proposed research question, the inclusion criteria were: (1) systematic review (with or without meta-analysis); (2) addressing sex determination using a forensic dentistry approach. No restrictions on language or publication year were applied.

2.2. Information Sources Search

Electronic data search was performed in four electronic databases: PubMed, Cochrane Database of Systematic Reviews, LILACS (Latin-American scientific literature in health sciences), and Web of Science up to December 2021. Keywords and subject headings were merged with the following syntax "((sex determination [MeSH terms]) OR (sex determination) OR (sex estimation) OR (gender estimation) OR (sex prediction) OR (canine index) OR (sexual dimorphism) OR (sex determination) OR (dental dimorphism) OR (gender determination) OR (sex dimorphism [MeSH terms]) OR (sex dimorphism)) AND (tooth OR teeth OR canine OR premolar OR molar) AND ((Systematic Review) OR (Meta-analysis))". Grey literature was also scanned.

2.3. Study Selection

Two researchers (J.A.N. and J.B.) separately screened titles and abstracts. The agreement between the reviewers was assessed by Kappa statistics. Any paper classified as potentially eligible by either reviewer was ordered as a full-text and independently screened. All disagreements were solved through discussion with a third reviewer (V.M.).

2.4. Data Extraction Process and Data Items

Two researchers (J.A.N. and J.B.) separately extracted: authors and year of publication, objective/focused question, databases searched, number of studies included, type of studies included, main results, and main conclusions. All disagreements were solved through discussion with a third reviewer (V.M.).

2.5. Risk of Bias and Methodological Quality Assessment

Two researchers (J.A.N. and J.B.) applied the measurement tool to assess systematic reviews (AMSTAR 2) to establish the methodological quality of the included reviews [16]. AMSTAR 2 is a comprehensive 16-item tool that rates the overall confidence of the results of the review. As stated in the AMSTAR guidelines, the quality of the systematic reviews was classified as follows: high means 'zero or one non-critical weakness'; moderate means 'more than one non-critical weakness'; low means 'one critical flaw with or without non-critical weaknesses'; and critically low means 'more than one critical flaw with or without non-critical weaknesses'. The estimation of the AMSTAR quality rate for each study was calculated through the AMSTAR 2 online tool (https://amstar.ca/Amstar_Checklist.php, accessed on 1 December 2021).

2.6. Synthesis of Results

We anticipated a heterogeneous variety of systematic reviews, and for this reason meta-analysis was deemed not possible to carry out. Yet, we described the results of each systematic review according to the type of procedure: dental, molecular, and artificial intelligence methods, following the SWiM guideline [17].

3. Results

3.1. Study Selection

Electronic searches redeemed a total of 370 titles through the database searching. Following manual assessment of title/abstract and removal of duplicates, 12 potentially eligible full texts were screened. Full-text screening excluded 7 studies with reasons, resulting in 5 systematic reviews that fulfilled the inclusion criteria (Figure 1). Inter-examiner reliability at the full-text screening was recorded as high (kappa score = 1.00).

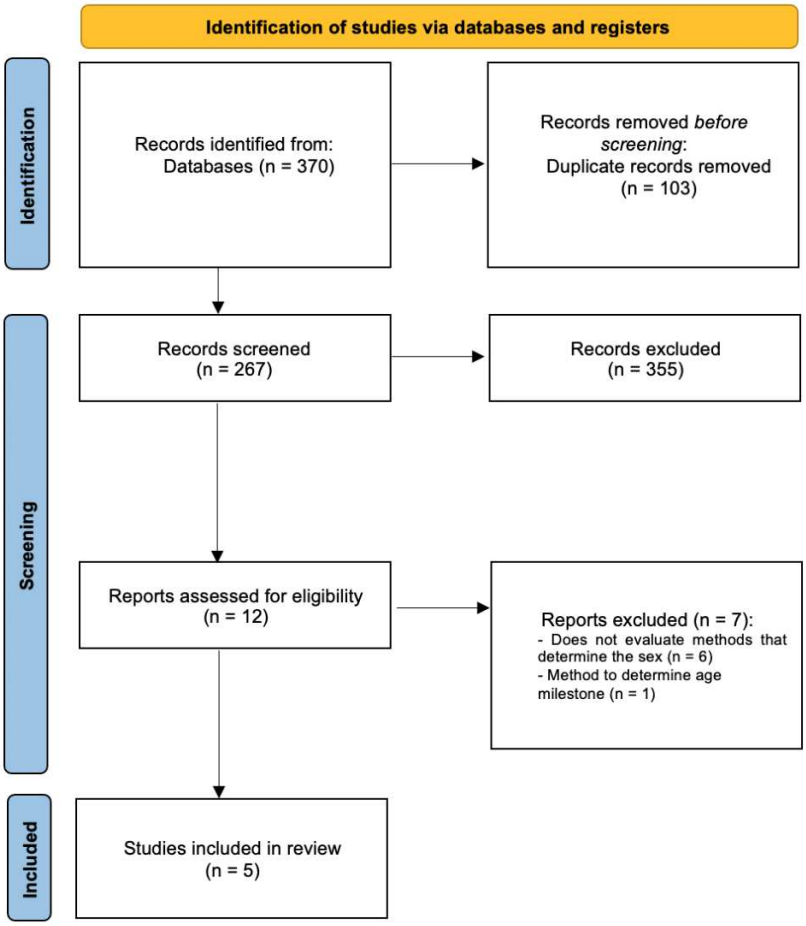


Figure 1. PRISMA flowchart of included studies.

3.2. Study Characteristics

In total, five systematic reviews were included in the present umbrella review (Table 1). All SRs covered a defined timeframe [14,18–21]. Three systematics reviews restricted their search to studies in English [18,20,21] while the remaining had no language restrictions [14,19].

Table 1. Characteristics of included SRs.

Authors (Year)	N	Search Period	Interventions	Quality Assessment Tool	Sample	Method of Analysis	Outcomes	AMSTAR2 Score *	Funding
Capitaneanu et al. (2017) [14]	103	Up to November 2016	Odontological sex estimation methods	QAS	NR	SR & MA	Accuracy	Low	NI
Khanagar et al. (2021) [18]	8	January 2000 to June 2020	AI-based models for sex determination, age estimation, and personal identification	QUADAS-2	NR	SR	Accuracy, sensitivity, specificity, ROC, AUC, ICC, PPV, NPV	Low	Research Grant
Maulani et al. (2020) [21]	10	2009 to 2019	DNA analysis methods	None	NR	SR	Accuracy	Critically Low	None
Pratapiene et al. (2016) [20]	11	January 2004 to April 2014	Canine mesiodistal measures	Cochrane Collaboration Tool	NR	SR & MA	Sexual dimorphism	Critically Low	NI
Silva et al. (2019) [19]	31	October 2015 to July 2016	Tooth crown mesiodistal measures	Criteria developed by the authors	NR	SR & MA	Sexual dimorphism	Low	NI

AI—artificial intelligence; DNA—deoxyribonucleic acid; MA—meta-analysis; N—number of included studies; NI—no information; NR—not reported; QAS—quality assessment tool. QUADAS—quality assessment and diagnostic accuracy tool; SR—systematic review. * Detailed information regarding the methodological quality assessment is present in Table 2.

Table 2. Methodological quality of the included SRs.

First Author	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Review Quality
Capitaneau et al. (2017) [14]	Y	PY	N	PY	Y	N	N	Y	PY/PY	N	Y/Y	N	N	Y	N	Y	Low
Silva et al. (2019) [19]	Y	PY	N	PY	Y	N	N	Y	PY/PY	N	Y/Y	N	N	Y	N	Y	Low
Khanagar et al. (2020) [18]	Y	PY	N	PY	Y	Y	N	Y	PY/PY	N	0/0	0	N	N	0	Y	Low
Maulani et al. (2020) [21]	N	N	N	PY	N	N	N	PY	N/N	N	0/0	0	N	N	0	Y	Critically Low
Pratapiene et al. (2016) [20]	Y	PY	N	N	N	N	N	Y	PY/PY	N	Y/Y	Y	Y	N	N	Y	Critically Low

0—No meta-analysis conducted, N—No, Y—Yes, PY—Partial Yes. 1. Are research questions and inclusion criteria included? 2. Were review methods established a priori? 3. Is there an explanation of the review authors' selection literature search strategy? 4. Did the review authors use a comprehensive literature search strategy? 5. Was study selection performed in duplicate? 6. Was data selection performed in duplicate? 7. Is the list of excluded studies and exclusions justified? 8. Is the description of the included studies in adequate detail? 9. Is there a satisfactory technique for assessing the risk of bias (RoB)? 10. Is there a report on the sources of funding for the studies included in the review? 11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results? 12. If meta-analysis was performed, did the review authors assess the potential impact of RoB? 13. Was RoB accounted for when interpreting/discussing the results of the review? 14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review? 15. If they performed quantitative synthesis, was publication bias performed? 16. Did the review authors report any potential sources of conflict of interest, including funding sources?

Methodological Quality

Two studies were rated as of critically low quality [20,21] and three as of low quality [14,18,19] (detailed in Table 2). None of the included SR completely fulfilled the AMSTAR2 checklist. Overall, SRs mostly failed on: describing their selection of the study designs for inclusion (100%, $n = 5$); providing a list of excluded studies with the respective reasons for exclusion (100%, $n = 5$); accounting for RoB in individual studies when interpreting/discussing the results of the review (80%, $n = 4$); performing data extraction in duplicate (80%, $n = 4$).

3.3. Synthesis of Results

3.3.1. Dental Methods

Overall, the level of evidence of the SRs regarding forensic tools based on dental methods was of low quality.

All human teeth exhibit a small degree of sexual dimorphism, with the canines and the second molars being the most dimorphic teeth [18–20]. Regarding canines mesiodistal

measures alone, maxillary left canines were reported with the lowest sexual dimorphism. On the other hand, both right and left mandibular canines presented the higher dimorphic degree [19,20]. When considering all teeth, premolars showed the smallest dimorphic level [18–20].

Although dental dimorphism occurs among different racial groups, the difference between measures is very substantial, which seems to have geographically relevance due to environmental and genetic factors [19]. Nevertheless, it seems impossible to identify a universal predictor value across all populations [19,20].

The accuracy of methods based on measurements on casts was estimated to be between 34.5% and 90% (one study reported 100% accuracy) [18–20].

Despite biochemical analysis being presented as the most precise method, it is not appropriate in the forensic practice as an odontological sex estimation method [14].

3.3.2. Molecular Analysis

The level of evidence on molecular analysis in forensic dentistry was of critically low quality.

Most samples considered were obtained from the dentin and the pulp because oral tissue remnants are a rich source for sex determination by DNA tracing, specially amelogenin, a protein of epithelial origin, and SRY or sex-determining region Y, a sex-typing based on the Y chromosome [21]. This type of sex determination could reach 100% of expected accuracy [21].

The available body and tissue remnants, as well as their level of degradation, drives the clinicians to make the appropriate methodological choice [21].

3.3.3. Artificial Intelligence (AI) Technology in Forensic Dentistry

The level of evidence on AI-based methods for sex prediction using dental measures was of low quality.

In forensic dentistry, AI demonstrates accuracy and precision identical to qualified examiners, prevents possible human errors, and is non-invasive [18]. AI is a promising tool for mass disasters, especially in victim identification although there is an absence of real-life testing and validation [18].

4. Discussion

This umbrella review unequivocally summarizes the evidence sourced in sex determination in forensic dentistry. The methodological quality of the included SRs ranged from critically low to low quality, and consequently the present knowledge is supported by low-confidence evidence-based studies.

In forensic dentistry, sex determination is a key element during the process of human identification. It facilitates this process because the pool of missing people would be half, which allows the matching operation to be sped up [14]. Several studies were published regrading sex determination methods. Capitaneanu et al. [14] suggested that a reliable method should present an accuracy of over 80%, and even then, in a case of negative forensic match in the first search, it should be succeeded by a second search for antemortem data in both sexes [14]. Most studies tested Indian populations [14,19,20] but it is also stated that there are differences between populations, rendering the need for larger and geographically wider samples [19]. Many studies focus on a young adult population in order to ascertain the presence of healthy teeth, removing factors like tooth wear, cavities, and fractures from dental measurements [14,19]. Almost all studies agree with the election of the canine as the most dimorphic tooth, followed by the second molar [14,19,20]. On the other hand, premolar teeth showed the smallest dimorphism [14]. Few studies use skeletal remains, probably related to the difficulty of gathering a large sample of intact bodies with a full set of teeth [14].

As previously asserted, teeth are the hardest tissue in the human body, remaining intact during a considerable time and becoming a premium source for DNA collection, which is the most reliable source in sex determination. Samples were mostly collected from dental

pulp, intraoral epithelial cells from swabs, toothbrushes, and saliva from dental prosthesis [21]. Age seems not to have an influence on sex determination via DNA analysis. DNA degradation took place in samples that underwent simulated environmental challenges, such as desiccation, incineration, submersion in saltwater, or burial. However, DNA can be successfully collected and processed for sex determination through DNA amplification techniques, such as polymerase chain reaction (PCR) [21]. The lack of standardization of the DNA sources and conditions prevents a correct interpretation of the results [21].

AI models mimic, most of all, the human brain, and its ability to create mathematical reasoning, problem-solving, and decision making. But to do so, these AI models must be taught and trained [18]. Concerning sex determination, AI was only applied to identify sexual dimorphism in canines using orthopantomography. The methods used were a multilayer perceptron and an artificial neural network, overperforming discriminant analysis and logistic regression, two standard sex determination methods [18]. AI will play a major role in forensic dentistry, not only in sex determination but also in several other fields, such as age determination, bitemarks, or lip prints [18].

With the purpose of improving the quality of research, the checklist from TRIPOD [22] should be followed. The research question should be clearer with special emphasis on selection of study designs and reasons for exclusion. It would also enrich future SRs if they took into account the RoB of individual studies as well as the number of authors that performed data extraction, to prevent bias.

Strengths and Limitations

This umbrella review benefits from its comprehensive review of the available SRs using a clear methodology. However, one limitation does need to be accounted for when interpreting the results. In each SR, the individual studies included were not explored. Thus, the conclusions of this review are based on the interpretation of the authors.

5. Conclusions

The current knowledge is supported by low-confidence evidence-based studies. Although DNA is the best source for sex determination, forensic dental methods for sex estimation present some reliability. This umbrella review reports the most common mistakes performed in SRs and will pave the way for more robust evidence-based research in the future.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jpm12050787/s1, Table S1: Prisma 2020 Checklist.

Author Contributions: Conceptualization, V.M. and J.B.; methodology, J.A.N.; validation, J.B., V.M., and L.P.; formal analysis, L.P.; investigation, J.A.N.; resources, A.S.D.; writing—original draft preparation, J.A.N.; writing—review and editing, J.J.M., N.A.-F., and A.Q.; supervision, J.J.M., A.S.D., N.A-F., and A.Q.; project administration, J.J.M. and A.S.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work is financed by national funds through the FCT—Foundation for Science and Technology, I.P., under project UIDB/04585/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Johnson, A. A Morphological Study of Tongue and Its Role in Forensics Odontology. *J. Forensic Sci. Crim. Investig.* **2018**, *7*, 1–5. [CrossRef]

2. Zorba, E.; Moraitis, K.; Manolis, S.K. Sexual Dimorphism in Permanent Teeth of Modern Greeks. *Forensic Sci. Int.* **2011**, *210*, 74–81. [CrossRef] [PubMed]
3. INTERPOL. Disaster Victim Identification Guide. In *INTERPOL DVI Guide Review*; Volume Guide & Annexures; INTERPOL: Lyon, France, 2018.
4. Angadi, P.V.; Hemani, S.; Prabhu, S.; Acharya, A.B. Analyses of Odontometric Sexual Dimorphism and Sex Assessment Accuracy on a Large Sample. *J. Forensic Leg. Med.* **2013**, *20*, 673–677. [CrossRef] [PubMed]
5. Khamis, M.F.; Taylor, J.A.; Malik, S.N.; Townsend, G.C. Odontometric Sex Variation in Malaysians with Application to Sex Prediction. *Forensic Sci. Int.* **2014**, *234*, 183.e1–183.e7. [CrossRef]
6. Mitsea, A.G.; Moraitis, K.; Leon, G.; Nicopoulou-Karayianni, K.; Spiliopoulou, C. Sex Determination by Tooth Size in a Sample of Greek Population. *HOMO J. Comp. Hum. Biol.* **2014**, *65*, 322–329. [CrossRef]
7. Silva, A.M.; Pereira, M.L.; Gouveia, S.; Tavares, J.N.; Azevedo, Á.; Caldas, I.M. A New Approach to Sex Estimation Using the Mandibular Canine Index. *Med. Sci. Law* **2016**, *56*, 7–12. [CrossRef]
8. Neves, J.A.; Antunes-Ferreira, N.; Machado, V.; Botelho, J.; Proença, L.; Quintas, A.; Mendes, J.J.; Delgado, S. Sex Prediction Based on Mesiodistalwidth Data in the Portuguese Population. *Appl. Sci.* **2020**, *10*, 4156. [CrossRef]
9. Brook, A.H.; Griffin, R.C.; Townsend, G.; Levisianos, Y.; Russell, J.; Smith, R.N. Variability and Patterning in Permanent Tooth Size of Four Human Ethnic Groups. *Arch. Oral Biol.* **2009**, *54*, S79–S85. [CrossRef]
10. Joseph, A.P.; Rajeesh Mohammed, P.K.; Kumar, V.R. How Reliable Is Sex Differentiation from Teeth Measurements. *Oral Maxillofac. Pathol. J.* **2013**, *4*, 289–292.
11. Basman, R.S.; Achmad, R.T.; Utari, D.R.; Harya Bima, T.R.A.; Auerkari, E.I. Types of Palatal Rugae for Sex Determination in the Indonesian Subpopulation. *J. Int. Dent. Med. Res.* **2019**, *12*, 1433–1435.
12. Cameriere, R.; Ferrante, L.; de Angelis, D.; Scarpino, F.; Galli, F. The Comparison between Measurement of Open Apices of Third Molars and Demirjian Stages to Test Chronological Age of over 18 Year Olds in Living Subjects. *Int. J. Leg. Med.* **2008**, *122*, 493–497. [CrossRef]
13. Roewer, L. Y-chromosome Short Tandem Repeats in Forensics—Sexing, Profiling, and Matching Male DNA. *WIREs Forensic Sci.* **2019**, *1*, e1336. [CrossRef]
14. Capitaneanu, C.; Willems, G.; Thevissen, P. A Systematic Review of Odontological Sex Estimation Methods. *J. Forensic Odontostomatol.* **2017**, *35*, 1–19.
15. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; Group, P. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *BMJ* **2009**, *339*, b2535. [CrossRef]
16. Burda, B.U.; Norris, S.L.; Holmer, H.K.; Ogden, L.A.; Smith, M.E.B. Quality Varies across Clinical Practice Guidelines for Mammography Screening in Women Aged 40–49 Years as Assessed by AGREE and AMSTAR Instruments. *J. Clin. Epidemiol.* **2011**, *64*, 968–976. [CrossRef]
17. Campbell, M.; McKenzie, J.E.; Sowden, A.; Katikireddi, S.V.; Brennan, S.E.; Ellis, S.; Hartmann-Boyce, J.; Ryan, R.; Shepperd, S.; Thomas, J.; et al. Synthesis without Meta-Analysis (SWiM) in Systematic Reviews: Reporting Guideline. *BMJ* **2020**, *368*. [CrossRef]
18. Khanagar, S.B.; Vishwanathaiah, S.; Naik, S.; Al-Kheraif, A.A.; Devang Divakar, D.; Sarode, S.C.; Bhandi, S.; Patil, S. Application and Performance of Artificial Intelligence Technology in Forensic Odontology—A Systematic Review. *Leg. Med.* **2021**, *48*, 101826. [CrossRef]
19. Da Silva, P.R.; Lopes, M.C.; Martins-Filho, I.E.; Haye Biazevic, M.G.; Michel-Crosato, E. Tooth Crown Mesiodistal Measurements for the Determination of Sexual Dimorphism across a Range of Populations: A Systematic Review and Meta-Analysis. *J. Forensic Odontostomatol.* **2019**, *37*, 2–19.
20. Pratapiene, M.; CICCIù, M.; Juodzbalys, G. Canines Mesiodistal Measures as the Key to Sex Prediction: A Systematic Review and Meta-Analysis. *Minerva Pediatr.* **2016**, *68*, 288–298.
21. Maulani, C.; Auerkari, E.I. Molecular Analysis for Sex Determination in Forensic Dentistry: A Systematic Review. *Egypt. J. Forensic Sci.* **2020**, *10*, 1–9. [CrossRef]
22. Moons, K.G.M.; Altman, D.G.; Reitsma, J.B.; Ioannidis, J.P.A.; Macaskill, P.; Steyerberg, E.W.; Vickers, A.J.; Ransohoff, D.F.; Collins, G.S. Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD): Explanation and Elaboration. *Ann. Intern. Med.* **2015**, *162*, W1–W73. [CrossRef] [PubMed]

4.2. Evidence of age estimation procedures in Forensic Dentistry: results from an umbrella review

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Review

Evidence of Age Estimation Procedures in Forensic Dentistry: Results from an Umbrella Review

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Abstract: *Background and objective*: Age estimation is an important tool when dealing with human remains or undocumented minors. Although the skull, the skeleton or the hand-wrist are used in age estimation as maturity indicators, they often present a lack of good conditions for a correct identification or estimation. Few systematic reviews (SRs) have been recently published; therefore, this umbrella review critically assesses their level of evidence and provides a general, comprehensive view. *Materials and methods*: Considering the review question "What is the current evidence on age determination approaches in Forensic Dentistry?" an electronic database search was conducted in four databases (PubMed, Cochrane, WoS, LILACS) up to December 2022, focusing on SRs of age estimation through forensic dentistry procedures. The methodological quality was analyzed using the measurement tool to assess SRs criteria (AMSTAR2). *Results*: Eighteen SRs were included: five of critically low quality, six of low quality, three of moderate quality and four of high quality. The SRs posited that Willems' method is more accurate and less prone to overestimation; most methods seem to be geographically sensitive; and 3D-imaging and artificial intelligence tools demonstrate high potential. *Conclusions*: The quality of evidence on age estimation using dental approaches was rated as low to moderate. Well-designed clinical trials and high-standard systematic reviews are essential to corroborate the accuracy of the different procedures for age estimation in forensic dentistry.

Keywords: forensic dentistry; age estimation; dental maturation; umbrella review

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Citation: Neves, J.A.; Lopes, L.B.; Machado, V.; Botelho, J.; Delgado, A.S.; Mendes, J.J. Evidence of Age Estimation Procedures in Forensic Dentistry: Results from an Umbrella Review. *Medicina* **2024**, *60*, 42. https://doi.org/10.3390/medicina60010042

Received: 16 October 2023
Revised: 16 December 2023
Accepted: 21 December 2023
Published: 25 December 2023

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1. Introduction

Age estimation is a key forensic and archeological element. Often useful for forensic identification of human remains, legal assistance involving minors or clinical diagnosis and planning [1–4], it is also helpful in mass migration and lack of valid identification [1,2,5]. Several methods have been developed to this end, among them, skeletal and dental development, sexual maturation or height/weight ratios [6,7].

Although the skull, the skeleton or the hand-wrist are used in age estimation as maturity indicators, they often present a lack of good conditions for a correct identification or estimation [8,9]. Teeth are the hardest human organs and are often found in adequate conditions [10–12]. Furthermore, dental measurements and indices are considered more useful and reliable, due to less variability during development as well as the greater resistance of teeth to systemic, environmental or destructive factors [6,13].

Estimating dental age may be achieved through several strategies depending on whether tooth development (around 20 years of age) or body development have been completed. On the one hand, methodologies based on teeth development are more accurate and with a smaller margin of error [8,14]. On the other hand, the biological age of the individual is being estimated, always understood in a period of time, with some level of

precision, and according to the method used. Chronological age will be included, at best, in this age range [12,14].

Several systematic reviews have been published with numerous dental methods based on radiographic (panoramic radiographs or otherwise) and non-radiographic approaches, most of them only evaluate one or two methodologies. Considering the variety and the discrepancy of the methods, it is helpful to compare and summarize the evidence previously published regarding age determination in Forensic Dentistry. The purpose of this comprehensive review was to assess the existing evidence on age determination procedures in forensic dentistry. Our focus was twofold: to determine the quality of the evidence and the overall clinical accuracy of each procedure.

2. Materials and Methods

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline [15] (Supplementary File S1) and the guide for systematic reviews of systematic review [16]. The review protocol was approved a priori by all authors and registered on Open Science Framework (DOI 10.17605/OSF.IO/CPBZY).

The review question was: "What is the current evidence on age determination approaches in Forensic Dentistry?".

Eligibility criteria

To answer the proposed research question, the inclusion criteria were: (1) systematic review (with or without meta-analysis); (2) addressing age determination in Forensic Dentistry; (3) absence of data duplication within the included studies in the meta-analysis. No restrictions on the year of publication or language were applied.

Information sources search

Four electronic databases were searched for electronic data: PubMed, Cochrane Database of Systematic Reviews, LILACS (Latin American scientific literature in health sciences) and Web of Science. The key words and subject headings were merged in accordance with the thesaurus of each of the databases and the subject headings were exploded, with the following syntax "((age determination) OR (age determination forensic) OR (age estimation) OR (dental age estimation) OR (forensic age estimation) OR (age estimation methods) OR (age prediction) OR (dental age prediction)) AND ((tooth) OR (teeth) OR (dental) OR forensic OR (forensic dentistry) OR (forensic odontology)) AND ((Systematic Review) OR (Meta-analysis))". Grey literature searches were conducted in three appropriate databases (opensigle.inist.fr, https://www.ntis.gov/, https://www.apa.org/pubs/databases/psycextra, accessed on 22 November 2022).

Study selection

Two researchers (JAN and LBL) independently reviewed titles and abstracts. Agreement between the reviewers was assessed using kappa statistics. Any paper that was deemed to be potentially eligible by one of the two reviewers was ordered as a full-text article and screened independently by the reviewers. Disagreements were discussed with a third reviewer (JB).

Data extraction process and data items

Two reviewers (JAN and LBL) separately extracted the following: authors and year of publication, objective/focal question, databases scanned, number of studies included, type of studies included, main results and main conclusions. Any differences of opinion were resolved by discussion with a third reviewer (JB).

Risk of bias assessment

To determine the methodological quality of the included systematic reviews, two researchers (JAN and LBL) used the A Measurement Tool to Assess Systematic Reviews (AMSTAR 2) [16]. AMSTAR 2 is a comprehensive 16-item tool that ranks the overall methodological quality of a systematic review. Accordingly, the quality is ranked as follows: High

means 'Zero or one non-critical weakness'; Moderate means 'More than one non-critical weakness'; Low means 'One critical flaw with or without non-critical weaknesses'; and Critically low means 'More than one critical flaw with or without non-critical weaknesses. The AMSTAR 2 online tool was used to calculate the AMSTAR quality score for each study. (https://amstar.ca/Amstar_Checklist.php, accessed on 11 February 2023).

3. Results

Study selection

A total of 738 titles were identified by the electronic search. Thirty-four potentially eligible full texts were screened after manual assessment of the title/abstract and deletion of duplicates (Figure 1). During the full-text screening, 16 studies were excluded with justification (Supplementary File S2). A total of 18 systematic reviews met the inclusion criteria. The inter-rater reliability of the full-text screening process was found to be high (kappa score = 1.00).

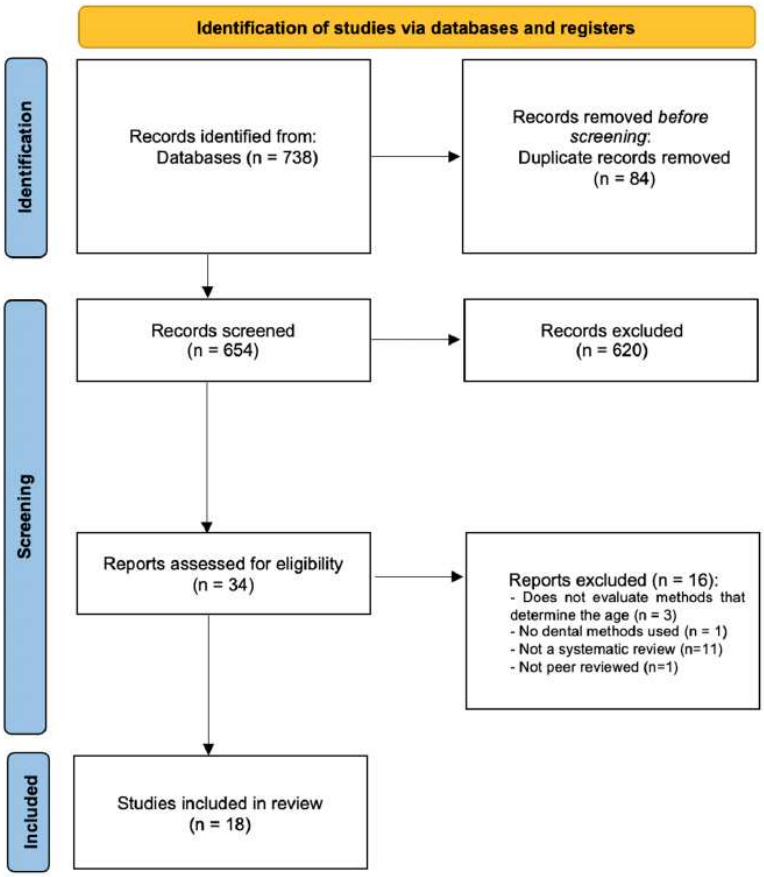


Figure 1. PRISMA flowchart of included studies.

SR characteristics

Overall, fourteen SRs [6,17–29] with meta-analysis and four without [30–33] were included (Table 1). Multiple sub-topics were investigated, such as imagiology methods based on panoramic X-rays [6,19–22,24,26], Cone Beam Computer Tomography (CBCT), Computer Tomography (CT) [30,32] and Magnetic Resonance Imaging (MRI) [17,30] (Table 1).

Table 1. Characteristics of included SRs.

Authors (Year)	N	Search Period	Interventions	Quality Assessment Tool	Sample	Method of Analysis	Outcomes	AMSTAR2 Score *	Funding
Bjork (2018) [30]	27	From July 2004 to September 2017	CT and MR imaging	NI	RCTs	SR	Although more research is needed, both CT and MR imaging may be useful tools for age estimation.	Critically Low	NI
De Tobel (2020) [17]	55	Up to September 2018	MR imaging	EPOC overview and QUADAS-2	1 prospective cohort; 35 prospective CS; 19 retrospective CS	SR/MA	The performance of the age estimation was better for the multi-factorial age estimation than for the single-site age estimation. MRI allows the examination of multiple anatomical sites without the use of ionizing radiation.	Moderate	NI
Diaconescu (2021) [18]	25	From 2013 to 2019	Chaillet's method	STROBE	RCTs	SR/MA	Chaillet's method showed age overestimation in both sexes, as in most ethnic groups, with delayed dental development in Asian populations, unlike European populations.	Low	NI
Esan (2017) [21]	28	Up to 28 December 2016	Demirjian's and Willems' methods	STROBE	5 comparative CS; 4 CS; 17 retrospective CS; 2 observational CS	SR/MA	While Demirjian's method has wide application in determining maturity scores, Willems' method provides a more accurate estimate of chronological age in different populations.	High	None
Franco (2020) [22]	13	Up to January 2019	Demirjian, Willems, Cameriere's, Nolla's and Lilequist and Lundberg's methods	JBI Critical Appraisal Tools	CS studies	SR/MA	Optimal performance was achieved by most of the international methods for dental radiographic age estimation.	Moderate	Research Grant
Haglund (2018) [23]	24	Up to 8 June 2017	Demirjian's method for the 3rd molar	QUADAS-2	RCTs	SR/MA	In addition to the fact that the presence of an immature third tooth is highly indicative of adult age, there are significant numbers of young adults (over the age of 18) with immature third teeth.	Critically Low	NI
Hostiuc (2021) [20]	89	From 1973 to 2020	Demirjian's method	STROBE	RCTs	SR/MA	Using the Demirjian method, the age is overestimated by about half a year for both sexes. Some geographic/ethnic differences exist. Nevertheless, regardless of the ethnic profile of the subjects, this method is useful.	Low	None

Table 1. *Cont.*

Authors (Year)	N	Search Period	Interventions	Quality Assessment Tool	Sample	Method of Analysis	Outcomes	AMSTAR2 Score *	Funding
Hostiuc (2021) [19]	15	From 2005 to 2019	Cameriere's method	STROBE	RCTs	SR/MA	At least in the 7–14 age interval, the Cameriere method is accurate enough for clinical use. However, it should not be used outside of this age range.	Low	None
Jayaraman (2013) [24]	34	From January 1973 to December 2011	Demirjian's method	NI	RCTs	SR/MA	Demirjian's method overestimates the age of the subjects by more than six months, and therefore this data set should only be used with great caution when trying to estimate the age of a group of subjects in any global population.	Critically Low	None
Khanagar et al. (2021) [31]	8	From January 2000 to June 2020	AI based models for personal age estimation	QUADAS-2	NR	SR	The AI technology demonstrates an accuracy and precision that is equivalent to that of a trained examiner, overcoming human error and being non-invasive are additional advantages of these models. A major limitation of the present review is the lack of real-life scenarios and the experimental nature of the included studies.	Low	Research Grant
Marroquin (2017) [32]	32	From January 1995 to July 2016	Cameriere's, Kvaal's method, and CBCT imaging	NI	NR	SR	More accurate results were obtained with age estimation methods based on pulp/tooth area ratio calculation. It is advised to use dental age estimation methods, firstly, pulp/tooth area ratio calculation of single first, upper canines and other single rooted teeth, and secondly, pulp/tooth length/ ratio calculation.	Critically Low	NI
Yusof (2017) [25]	23	From January 2001 to September 2014	Willems' method	Cochrane handbook for systematic reviews-methodology review	NR	SR/MA	Given the accuracy of the Willems method across different populations, investigators, and age groups, it is appropriate to use this method to estimate age in children.	Moderate	Research Grant

Table 1. *Cont.*

Authors (Year)	N	Search Period	Interventions	Quality Assessment Tool	Sample	Method of Analysis	Outcomes	AMSTAR2 Score *	Funding
Prasad (2019) [26]	20	Up to July 2018	Demirjian's and Willems' methods	QUADAS-2	NR	SR/MA	Willems' method was more accurate in predicting chronological age than Demirjian's method in the Indian population, regardless of sex.	High	NI
Rolseth (2018) [33]	21	Up to May 2016	Demirjian's method for the 3rd molar	QUADAS-2	NR	SR	The differences in the timing of the developmental stages of the third molars according to Demirjian have often been interpreted as differences between populations and ethnic groups.	Low	None
Santiago (2017) [27]	15	Up to November 2017	Cameriere's method (I3M)	QUADAS-2	CS studies	SR/MA	The Third Molar Maturity Index is a suitable and useful method for estimating adulthood because it has a high accuracy in distinguishing whether an individual has reached 18 years of age, regardless of the population studied.	High	None
Sehrawat (2017) [28]	31	From 2001 to January 2017	Willems' method	NI	CS and Retrospective studies	SR/MA	Compared to other methods reported in the available literature, the Willems method of dental age estimation results in comparatively less overestimation of age.	Critically Low	NI
Wang (2017) [29]	11	Up to 28 February 2017	Willems' method	NOS	CS and Retrospective studies	SR/MA	For both sexes, the Willems method overestimated dental age between 3.0 and 16.9 years in almost all age groups. In addition, the accuracy of the Willems method was also shown to be affected by ethnic differences.	Low	NI
Yan (2013) [6]	26	Up to 12 July 2013	Demirjian's method	STROBE	CS and Retrospective studies	SR/MA	The fact that Demirjian's method overestimates actual chronological tooth age highlights the need for population-specific standards to better estimate the rate at which human teeth mature.	High	None

AI—artificial intelligence; CBCT—cone beam computer tomography; CS—Cross-sectional; CT-computer tomography, EPOC—effective practice and organization of Care; JBI—Joanna Briggs Institute; MA—meta-analysis; MR- magnetic resonance; N—number of included studies; NI—no information; NOS—Newcastle-Ottawa Scale; NR—not reported; QAS—quality assessment tool. QUADAS—quality assessment and diagnostic accuracy tool; RCT—randomized controlled trials; SR—systematic review; STROBE-Strengthening the reporting of observational studies in epidemiology. * Detailed information regarding the methodological quality assessment is present in Table 2.

Table 2. Methodological quality of the included SRs.

First Author	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Review Quality
Bjørk et al. (2018) [30]	Y	PY	N	N	N	N	N	N	0/0	N	0/0	0	Y	N	0	Y	Critically low
De Tobel et al. (2020) [17]	Y	PY	Y	Y	Y	Y	Y	PY	0/0	N	0/0	0	Y	Y	0	Y	Moderate
Diaconescu et al. (2021) [18]	Y	PY	Y	PY	N	N	PY	N	N/0	N	Y/0	Y	Y	Y	Y	Y	Low
Esan et al. (2017) [21]	Y	Y	Y	PY	Y	Y	Y	PY	PY/PY	N	Y/Y	Y	Y	Y	Y	Y	High
Franco et al. (2020) [22]	Y	Y	Y	PY	N	N	Y	N	PY/PY	N	Y/Y	Y	Y	Y	Y	Y	Moderate
Hadlund et al. (2018) [23]	Y	PY	Y	PY	N	N	Y	PY	N/N	N	Y/Y	Y	Y	Y	Y	Y	Critically low
Hostiuc et al. (2021) [20]	Y	Y	Y	N	Y	Y	Y	PY	N/0	N	Y/0	Y	Y	Y	Y	Y	Critically low
Hostiuc et al. (2021) [19]	Y	PY	N	Y	Y	Y	PY	N	N/0	N	Y/0	Y	Y	Y	Y	Y	Low
Jayaraman et al. (2013) [24]	Y	N	N	N	N	N	Y	PY	N/0	N	N/0	N	N	N	N	Y	Critically low
Khanagar et al. (2020) [31]	Y	PY	N	PY	Y	Y	N	Y	PY/PY	N	0/0	0	N	N	0	Y	Low
Marroquin et al. (2017) [32]	Y	PY	N	PY	N	N	Y	PY	N/N	N	0/0	0	N	N	0	N	Critically low
Mohd Yusof et al. (2017) [25]	Y	Y	N	PY	Y	N	Y	PY	Y/Y	N	Y/Y	Y	Y	Y	Y	Y	Moderate
Prasad et al. (2019) [26]	Y	Y	Y	Y	Y	Y	PY	Y	Y/Y	N	Y/Y	Y	Y	Y	Y	Y	High
Rolseth et al. (2018) [33]	N	Y	N	PY	Y	Y	N	PY	Y/Y	N	0/0	0	Y	Y	0	Y	Low
Santiago et al. (2017) [27]	Y	Y	Y	Y	Y	Y	Y	Y	Y/Y	N	Y/Y	Y	Y	Y	Y	Y	High
Sehrawat et al. (2017) [28]	Y	PY	Y	PY	Y	N	PY	Y	N/N	N	Y/Y	N	N	Y	N	Y	Low
Wang et al. (2017) [29]	Y	Y	Y	PY	Y	Y	PY	Y	Y/Y	N	Y/Y	Y	Y	Y	N	Y	Low
Yan et al. (2013) [6]	Y	Y	Y	Y	Y	Y	Y	Y	Y/Y	N	Y/Y	Y	Y	Y	Y	Y	High

0—No meta-analysis conducted, N—No, Y—Yes, PY—Partial Yes. 1. Are research questions and inclusion criteria included? 2. Were review methods established a priori? 3. Is there an explanation of the review authors' selection literature search strategy? 4. Did the review authors use a comprehensive literature search strategy? 5. Was study selection performed in duplicate? 6. Was data selection performed in duplicate? 7. Is the list of excluded studies and exclusions justified? 8. Is the description of the included studies in adequate detail? 9. Is there a satisfactory technique for assessing the risk of bias (RoB)? 10. Is there a report on the sources of funding for the studies included in the review? 11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results? 12. If meta-analysis was performed, did the review authors assess the potential impact of RoB? 13. Was RoB accounted for when interpreting/discussing the results of the review? 14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review? 15. If they performed quantitative synthesis, was publication bias performed? 16. Did the review authors report any potential sources of conflict of interest, including funding sources?

One SR failed to report a defined timeframe [33]. Seven SRs described their studies eligibility criteria in the review [20,24,25,30–33], while four could not describe in adequate detail [18,19,22,30], and eight had no funding information [17,18,23,26,28–30,32].

Methodological Quality

We observed excellent inter-examiner reliability at the RoB assessment (kappa score = 0.93; 95% confidence interval: 0.91–0.95).

None of the included SRs fully satisfied the AMSTAR2 Criteria (Table 2). Four studies were rated as of 'high quality' [6,21,26,27], three as of 'moderate quality' [17,22,25], six as of 'low quality' [18,20,28,29,31,33] and five as of 'critically low quality' [19,23,24,30,32].

All studies did not report the sources of funding for the studies included in the review, when analyzing the major inconsistencies identified by AMSTAR2 (100%, n = 18) [6,17–33]; 44.4% did not have data selection in duplicate (n = 8) [18,22–25,28,30,32]; 38.9% of studies did not explain selection literature search strategy (n = 7) [19,24,25,30–33]; and 33.3% did not have study selection in duplicate (n = 6) [18,22–24,30,32].

Synthesis of Results

Overall, three main topics of research were found among the included SRs: panoramic radiographs-based methods; three-dimensional imaging methods; and artificial intelligence (AI)-based methods.

Panoramic radiographs-based methods

Overall, the level of evidence of the SRs focusing forensic methods based on panoramic radiographs was of low quality. Three main methods were the aim of research: Demirjian's [6,20–24,26,33], Willems' [21,22,25,26,28,29] and Cameriere's [19,22,27,32].

As regards to Demirjian's method, all studies are in agreement of an overestimation that varies between 4 to 9 months [6,20–22,24,26]. The majority affirm that this method is geographically sensitive [6,20,21,24], except the studies that are single-population oriented [22,26]. With respect to sex, two studies tend to overestimate the females [21,24], two showed more overestimation in males and lastly, one study reported that there were no differences between sexes [6] and one did not analyze sex subgroups [22]. Demirjian's method was also applied solely to the third molar in two papers. Haglund et al. [23] defined the accuracy of the method for the 18 years old threshold as 71% and Rolseth et al. [33] identified that the different development ranged from 4 to 7 years of the 3rd molar mineralization.

As for the Willems' method, most studies showed a slight age overestimation, varying between 1 to 5 months [21,22,25,28,29], aside from one [26], that concluded underestimation by a month. They also conclude that males are more susceptible to this overestimation [25,26,28,29], except one [21], which concludes that females are more sensitive. One other study [22] does not analyze sex subgroups. Respecting age and geographic subgroups, some authors [21,25,28,29] reported differences between populations as the rest only studied a single population [22,26]; Esan et al., Yosuf et al. and Wang et al., also stated that a few age subgroups are more prone to overestimation [21,25,29].

Regarding Cameriere's method, studies demonstrated overestimation that varied from 3 months to one year, making this variation slightly greater in males, but without statistically significant difference [19,22,32]. Marroquin et al. [32] also reported that in the Indian subpopulation, this variation can be as high as 10 years of overestimation. Cameriere also developed an index to assess the threshold of 18 years old with a percentage of correct classification ranging from 72 to 96%, with a better accuracy in males [27].

Kvall's method [32] overestimates age between 1 to 2 and 12 to 13 years old and Chaillet's method [18] within 6 to 8 months. Both methods overestimate more females and present different results when analyzing various geographical subpopulations.

Other methods included in this overview were only investigated by Franco et al. [22] for the Brazilian population. Nolla's method demonstrated 2 to 3 months of overestimation, Lilequist and Lundberg's method 1 to 2 months of underestimation, Mornstard's 3 to 4 months of overestimation and lastly, Haavikko's method underestimates between 10 to 12 months.

Three-dimensional imaging methods

Overall, the level of evidence of the SRs relating to forensic methods based on 3-dimensional imaging was of low quality.

Cone Beam Computed Tomography (CBCT) and Computed Tomography (CT) Scans reported different margin of errors, according to the method applied, ranging from 3,5 to 28 years [32]. When using pulp/tooth ratio, individuals were correctly identified between

30 and 90%, being the majority around 60% [30]. Both studies [30,32] agree that these values differ depending on the sex of the individual and the type of the tooth.

As for Magnetic Resonance Imaging (MRI), all studies concluded that results from images obtained through MRI are equated to panoramic radiographs, without the ionizing radiation [17,30].

AI-based methods

The level of evidence on AI-based methods for sex prediction using dental measures was collectively based on low quality SRs. AI displayed precision and accuracy similar to trained examiners, overcoming the observer subjectivity. Although the accuracy, real-life testing and validation are yet to be proved [31].

4. Discussion

This umbrella review was able to sum up the evidence provided by the available SRs on age estimation methods in Forensic Dentistry. The collective knowledge is currently based on low- to moderate-confidence evidence-based studies, at best ranging from critically low to high quality. Overall, these results show that, due to the poor quality of many of the studies mentioned here, some of the forensic tools used today may be outdated or misused, and some results must be analyzed with caution.

Age estimation is a major step in forensic and archeological investigations [4]. Despite several methods that have been developed to this end, approaches using dental tissues are among the most useful and reliable due to their low changeability and greater resistance to degradation [7,13]. Most studies rely on methods developed upon bidimensional radiographic images, such as Demirjian's and Willems' methods, which have seen validation worldwide [6,18–22,24–29,32,33]. Both methods tend to overestimate age [6,20–26,28,29,33]. Cameriere's method was first studied for several age groups and later as an index (I3M) that differentiates the legal threshold of 18 years old [19,22,27,32]. Other methods included are Chaillet's, Lundberg's, Nolla's, Mornstad's, Haavik's and Kval's methods [18,22,32]. More recently, a few studies investigated the applicability of 3-dimensional imaging in forensic dentistry [17,30,32]. AI technology has already been tested for age estimation and seems like a potential forensic tool [31].

Demirjian's method obtained global acceptance and became the most widely used method for dental age estimation [21]. Nonetheless, most studies concluded that this method is geographically sensitive and varies according to the subpopulation studied [6,20,21,24]. A possible reason for such a thing was the origin of this dataset (Caucasian subpopulation) with low heterogeneity, different from the other subpopulations studied [6,20,21,24]. Demirjian's method tends to overestimate the age of the individuals, regardless of the sex of the subject. For such reasons, Demirjian's method renders as a poor forensic tool when misapplied [20,24]

Willems' method is also geographically sensitive [21,25,28,29]. Sehrawat et al. [28], for instance, determined that this method overestimates in the majority of the countries, except China and India. Sex seems to be a factor to consider, because this method tends to overestimate more males than females [21,25,26,28,29].

Three studies comparing both Demirjian and Willems' methods are all in agreement that the latter is more accurate and less prone to overestimation [21,22,26].

Demirjian's method was introduced in the 1970's and Willem's method in the 2000's. Since these methods (and most of the remaining methods) are based on tooth maturation and this characteristic is growth dependent, it is likely that it will need to be updated on a regular basis. Growth patterns have evolved with the improvement of healthcare, nutrition and genetics so new methods must be developed to accompany the evolution of times [21,34].

Not only in Forensic Dentistry, dental methods and indexes have been misused. In Orthodontics, indexes such as Bolton, developed from a specific subpopulation, have been proven incorrect when generalized to other populations [35].

Cameriere's method is one of the geographically stable methods and tends to overestimate by 4 months, both boys and girls, without statistically significant difference [19,22,32].

According to Hostiuc et al. this method seems to outperform several others, including Dejirmijian and Willems methods [19].

Santiago et al. concluded that the I3M has been validated in several sub-populations throughout the world, since it has a high accuracy in discriminating whether a person has reached the age of 18 years, regardless of the population studied. In terms of sex, men tend to have better results, but women also have high accuracy, sensitivity and specificity. [27].

Forensic age estimation based on dental measures has been, until recently, based on bidimensional imaging. Three-dimensional imaging is gaining more relevance in all dentistry areas, including forensic dentistry with the first study that reported the use of this technology was published in 2004 [36]. CBCT and CT scans reconstructions allow the investigators to analyze the pulp/tooth volume ratio. The volume ratio might be an interesting tool for predicting age after root maturation of the third molar, around the second decade of life [30]. Despite Micro-CT scans requiring the use of extracted teeth, the images are of greater quality and produce accurate measures because of more spatial resolution that of a CBCT; however, its application in live subjects is not viable, and a model based in this type of imaging may not be replicable for forensic proposes in live individuals [30,32]. Major limitations of this method are the artefacts produced by adjacent metal structures and restorations, such as implants and amalgam fillings [30]. Moreover, the difficulty in reproduction of the site for measurements might lead to an inaccurate analysis; the lack of a simple method of investigation should be the focus for the next researchers [30].

Due to the ethical implications of the usage of ionized radiation for other than diagnostic indications, MRI arose as a valid alternative since it uses strong magnetic fields and radio waves to generate imagens. It is a relatively new tool in age estimation, published for the first time in 2015 [37]. Regarding the methodology itself, MRI can be used associated with other methods previously stated, such as Demirjian's. MRI tends to be more accurate in the early stages of development but to be more challenging and inaccurate in the latter stages because of the lack of contract between dental and bone tissue. It also takes more time and is more expensive than an ordinary panoramic radiograph [30]. Due to the scarcity of research on this subject, the inter-ethnic variability is not yet proven [17]. Discrepancies between the MRI approaches make it inappropriate to pool data together and perform a proper systematic review with meta-analysis. Furthermore, future age estimation methods based on MRI will probably be based on multifactorial sites and measures [17].

AI-based automated systems have been developed to surpass the examiner's subjectivity. The AI model that best performed was the Deep Learning Convolutional Neural Network approach, with similar accuracy when compared with trained researchers. AI models that combine a dual Convolutional Neural Network, first to predict sex and then age, outperform a single Convolutional Neural Network approach. However, AI-based models have not proven themselves in the field to be routinely applied [31].

The TRIPOD [38] checklist should be followed for the improvement of research quality. Particular attention should be paid to the choice of study design and reasons for exclusion, and the research question should be more clearly formulated. In order to avoid biases, future SRs should consider the RoB of individual trials and the number of authors who performed data extraction.

Strengths and limitations

There are several strengths of the present umbrella review. Overall, using a transparent and evidence-based methodology, these results provide a comprehensive overview of the available SRs for age determination in Forensic Dentistry. Because the individual studies included in each of the present SRs were not reviewed, we recommend a cautious interpretation. Therefore, the conclusions rely on the interpretation of the authors of the systematic review.

5. Conclusions

Current evidence on forensic dentistry methods for age estimation is supported by low- to moderate-confidence systematic reviews. Willems' method is more accurate and less prone to overestimation. Most methods seem to be geographically sensitive, despite some authors attributing this heterogeneity to methodological errors. Cameriere's index has high accuracy, regardless of the population studied. Three-dimensional imaging and AI technology, although on the rise, still lack field validation. This umbrella review reports the most common mistakes performed in SRs and will pave the way for more robust evidence-based research in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/medicina60010042/s1, Table S1: PRISMA 2020 Checklist; Table S2: Detailed list of excluded articles with reasons.

Author Contributions: Conceptualization, V.M. and J.B.; methodology, J.A.N. and L.B.L.; validation, J.B. and V.M.; formal analysis, J.B.; investigation, J.A.N. and L.B.L.; resources, A.S.D.; writing—original draft preparation, J.A.N.; writing—review and editing, J.J.M. and A.S.D.; supervision, J.J.M. and A.S.D.; project administration, J.J.M. and A.S.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work is financed by national funds through the FCT—Foundation for Science and Technology, I.P., under project UIDB/04585/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cameriere, R.; Pacifici, A.; Viva, S.; Carbone, D.; Pacifici, L.; Polimeni, A. Adult or Not? Accuracy of Cameriere's Cut-off Value for Third Molar in Assessing 18 Years of Age for Legal Purposes. *Minerva Stomatol.* **2014**, *63*, 283–294. [PubMed]
2. Cunha, E.; Baccino, E.; Martrille, L.; Ramsthaler, F.; Prieto, J.; Schuliar, Y.; Lynnerup, N.; Cattaneo, C. The Problem of Aging Human Remains and Living Individuals: A Review. *Forensic Sci. Int.* **2009**, *193*, 1–13. [CrossRef] [PubMed]
3. Ritz-Timme, S.; Cattaneo, C.; Collins, M.J.; Waite, E.R.; Schütz, H.W.; Kaatsch, H.J.; Borrman, H.I. Age Estimation: The State of the Art in Relation to the Specific Demands of Forensic Practise. *Int. J. Leg. Med.* **2000**, *113*, 129–136. [CrossRef] [PubMed]
4. Schmeling, A.; Geserick, G.; Reisinger, W.; Olze, A. Age Estimation. *Forensic Sci. Int.* **2007**, *165*, 178–181. [CrossRef] [PubMed]
5. Angelakopoulos, N.; de Luca, S.; Velandia Palacio, L.A.; Coccia, E.; Ferrante, L.; Cameriere, R. Third Molar Maturity Index (I3M) for Assessing Age of Majority: Study of a Black South African Sample. *Int. J. Leg. Med.* **2018**, *132*, 1457–1464. [CrossRef] [PubMed]
6. Yan, J.; Lou, X.; Xie, L.; Yu, D.; Shen, G.; Wang, Y. Assessment of Dental Age of Children Aged 3.5 to 16.9 Years Using Demirjian's Method: A Meta-Analysis Based on 26 Studies. *PLoS ONE* **2013**, *8*, e84672. [CrossRef] [PubMed]
7. Mani, S.A.; Naing, L.; John, J.; Samsudin, A.R. Comparison of Two Methods of Dental Age Estimation in 7–15-Year-Old Malays. *Int. J. Paediatr. Dent.* **2008**, *18*, 380–388. [CrossRef]
8. Willems, G. A Review of the Most Commonly Used Dental Age Estimation Techniques. *J. Forensic Odontostomatol.* **2001**, *19*, 9–17.
9. Priyadarshini, C.; Puranik, M.P.; Uma, S.R. *Dental Age Estimation Methods: A Review*; Lambert Academic Publisher: Saarbrücken, Germany, 2015.
10. Capitaneanu, C.; Willems, G.; Thevissen, P. A Systematic Review of Odontological Sex Estimation Methods. *J. Forensic Odontostomatol.* **2017**, *35*, 1–19.
11. Peckmann, T.R.; Logar, C.; Garrido-Varas, C.E.; Meek, S.; Pinto, X.T. Sex Determination Using the Mesio-Distal Dimension of Permanent Maxillary Incisors and Canines in a Modern Chilean Population. *Sci. Justice* **2016**, *56*, 84–89. [CrossRef]
12. Angadi, P.V.; Hemani, S.; Prabhu, S.; Acharya, A.B. Analyses of Odontometric Sexual Dimorphism and Sex Assessment Accuracy on a Large Sample. *J. Forensic Leg. Med.* **2013**, *20*, 673–677. [CrossRef] [PubMed]
13. Maber, M.; Liversidge, H.M.; Hector, M.P. Accuracy of Age Estimation of Radiographic Methods Using Developing Teeth. *Forensic Sci. Int.* **2006**, *159*, S68–S73. [CrossRef] [PubMed]
14. Jeon, H.-M.; Jang, S.-M.; Kim, K.-H.; Heo, J.-Y.; Ok, S.-M.; Jeong, S.-H.; Ahn, Y.-W. Dental Age Estimation in Adults: A Review of the Commonly Used Radiological Methods. *J. Oral Med. Pain* **2014**, *39*, 119–126. [CrossRef]
15. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G. PRISMA Group Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med.* **2009**, *6*, e1000097. [CrossRef] [PubMed]

16. Burda, B.U.; Norris, S.L.; Holmer, H.K.; Ogden, L.A.; Smith, M.E.B. Quality Varies across Clinical Practice Guidelines for Mammography Screening in Women Aged 40–49 Years as Assessed by AGREE and AMSTAR Instruments. *J. Clin. Epidemiol.* **2011**, *64*, 968–976. [CrossRef] [PubMed]
17. de Tobel, J.; Bauwens, J.; Parmentier, G.I.L.; Franco, A.; Pauwels, N.S.; Verstraete, K.L.; Thevissen, P.W. Magnetic Resonance Imaging for Forensic Age Estimation in Living Children and Young Adults: A Systematic Review. *Pediatr. Radiol.* **2020**, *50*, 1691–1708. [CrossRef] [PubMed]
18. Diaconescu, I.; Isailă, O.-M.; Hostiuc, S. Accuracy of the Chaillet's Method for Assessing the Age in Subadults. A Meta-Analysis. *Curr. Health Sci. J.* **2021**, *47*, 196–203. [CrossRef]
19. Hostiuc, S.; Diaconescu, I.; Rusu, M.C.; Negoi, I. Age Estimation Using the Cameriere Methods of Open Apices: A Meta-Analysis. *Healthcare* **2021**, *9*, 237. [CrossRef]
20. Hostiuc, S.; Edison, S.E.; Diaconescu, I.; Negoi, I.; Isaila, O.M. Accuracy of the Demirjian's Method for Assessing the Age in Children, from 1973 to 2020. A Meta-Analysis. *Leg. Med.* **2021**, *52*, 101901. [CrossRef]
21. Esan, T.A.; Yengopal, V.; Schepartz, L.A. The Demirjian versus the Willems Method for Dental Age Estimation in Different Populations: A Meta-Analysis of Published Studies. *PLoS ONE* **2017**, *12*, e0186682. [CrossRef]
22. Franco, A.; de Oliveira, M.N.; Vidigal, M.T.C.; Blumenberg, C.; Pinheiro, A.A.; Paranhos, L.R. Assessment of Dental Age Estimation Methods Applied to Brazilian Children: A Systematic Review and Meta-Analysis. *Dentomaxillofac. Radiol.* **2021**, *50*, 20200128. [CrossRef] [PubMed]
23. Haglund, M.; Mörnstad, H. A Systematic Review and Meta-Analysis of the Fully Formed Wisdom Tooth as a Radiological Marker of Adulthood. *Int. J. Leg. Med.* **2019**, *133*, 231–239. [CrossRef] [PubMed]
24. Jayaraman, J.; Wong, H.M.; King, N.M.; Roberts, G.J. The French-Canadian Data Set of Demirjian for Dental Age Estimation: A Systematic Review and Meta-Analysis. *J. Forensic Leg. Med.* **2013**, *20*, 373–381. [CrossRef] [PubMed]
25. Mohd Yusof, M.Y.P.; Wan Mokhtar, I.; Rajasekharan, S.; Overholser, R.; Martens, L. Performance of Willem's Dental Age Estimation Method in Children: A Systematic Review and Meta-Analysis. *Forensic Sci. Int.* **2017**, *280*, 245.e1–245.e10. [CrossRef] [PubMed]
26. Prasad, H.; Kala, N. Accuracy of Two Dental Age Estimation Methods in the Indian Population—A Meta-Analysis of Published Studies. *J. Forensic Odontostomatol.* **2019**, *3*, 2–11.
27. Santiago, B.M.; Almeida, L.; Cavalcanti, Y.W.; Magno, M.B.; Maia, L.C. Accuracy of the Third Molar Maturity Index in Assessing the Legal Age of 18 Years: A Systematic Review and Meta-Analysis. *Int. J. Legal Med.* **2018**, *132*, 1167–1184. [CrossRef]
28. Sehrawat, J.S.; Singh, M. Willems Method of Dental Age Estimation in Children: A Systematic Review and Meta-Analysis. *J. Forensic Leg. Med.* **2017**, *52*, 122–129. [CrossRef]
29. Wang, J.; Ji, F.; Zhai, Y.; Park, H.; Tao, J. Is Willems Method Universal for Age Estimation: A Systematic Review and Meta-Analysis. *J. Forensic Leg. Med.* **2017**, *52*, 130–136. [CrossRef]
30. Bjørk, M.B.; Kvaal, S.I. CT and MR Imaging Used in Age Estimation: A Systematic Review. *J. Forensic Odontostomatol.* **2018**, *36*, 14–25.
31. Khanagar, S.B.; Vishwanathaiah, S.; Naik, S.; Al-Kheraif, A.A.; Devang Divakar, D.; Sarode, S.C.; Bhandi, S.; Patil, S. Application and Performance of Artificial Intelligence Technology in Forensic Odontology—A Systematic Review. *Leg. Med.* **2021**, *48*, 101826. [CrossRef]
32. Marroquin, T.Y.; Karkhanis, S.; Kvaal, S.I.; Vasudavan, S.; Kruger, E.; Tennant, M. Age Estimation in Adults by Dental Imaging Assessment Systematic Review. *Forensic Sci. Int.* **2017**, *275*, 203–211. [CrossRef] [PubMed]
33. Rolseth, V.; Mosdøl, A.; Dahlberg, P.S.; Ding, Y.; Bleka, Ø.; Skjerven-Martinsen, M.; Straumann, G.H.; Delaveris, G.J.M.; Vist, G.E. Age Assessment by Demirjian's Development Stages of the Third Molar: A Systematic Review. *Eur. Radiol.* **2019**, *29*, 2311–2321. [CrossRef] [PubMed]
34. Norris, S.A.; Frongillo, E.A.; Black, M.M.; Dong, Y.; Fall, C.; Lampl, M.; Liese, A.D.; Naguib, M.; Prentice, A.; Rochat, T.; et al. Nutrition in Adolescent Growth and Development. *Lancet* **2022**, *399*, 172–184. [CrossRef] [PubMed]
35. Machado, V.; Botelho, J.; Pereira, D.; Vasques, M.; Fernandes-Retto, P.; Proença, L.; Mendes, J.J.; Delgado, A. Bolton Ratios in Portuguese Subjects among Different Malocclusion Groups. *J. Clin. Exp. Dent.* **2018**, *10*, e864–e868. [CrossRef]
36. Vandevoort, F.M.; Bergmans, L.; van Cleynenbreugel, J.; Bielen, D.J.; Lambrechts, P.; Wevers, M.; Peirs, A.; Willems, G. Age Calculation Using X-ray Microfocus Computed Tomographical Scanning of Teeth: A Pilot Study. *J. Forensic Sci.* **2004**, *49*, 787–790. [CrossRef]
37. Baumann, P.; Widek, T.; Merkens, H.; Boldt, J.; Petrovic, A.; Urschler, M.; Kirnbauer, B.; Jakse, N.; Scheurer, E. Dental Age Estimation of Living Persons: Comparison of MRI with OPG. *Forensic Sci. Int.* **2015**, *253*, 76–80. [CrossRef]
38. Moons, K.G.M.; Altman, D.G.; Reitsma, J.B.; Ioannidis, J.P.A.; Macaskill, P.; Steyerberg, E.W.; Vickers, A.J.; Ransohoff, D.F.; Collins, G.S. Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD): Explanation and Elaboration. *Ann. Intern. Med.* **2015**, *162*, W1–W73. [CrossRef]

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CHAPTER 5

5. General Discussion, Concluding Remarks And Future Directions

5.1. General Discussion

The overall objective of this thesis was to develop and validate new tools and models for the Portuguese population regarding forensic dentistry, using dental measures, whether based on orthopantomographies, dental casts or any other source of dental origin. We not only identified a stepwise adjusted logistic regression procedure specific to our population that outperformed any previously published, but also validated one of the most established indices worldwide for the Portuguese population. We also assessed the level and quality of the current literature with respect to our stated overall objective.

To achieve this purpose, we have divided our overall objective into two phases. First, we analyzed a large sample of dental casts and orthopantomographies from the Egas Moniz University Clinic (Papers I and II). Thus, a large data set from a Portuguese population was analyzed, providing new results and answers. Furthermore, the umbrella reviews (Papers III and IV) carried out on the current evidence regarding the determination and prediction of sex and age in forensic dentistry, allow us to understand the lack of quality and the danger of poorly conducted systemic reviews (93,94).

As previously mentioned, in the Portuguese population, models for the estimation of sex through dental hard tissues are scarce, and the models proposed thus far lack consistency and consider only a small subset of teeth. Therefore, we aimed to develop a potential model based on the mesiodistal width of cast models previously studied for orthodontic indexes (Paper1). We then compared the performance of this model with other full mouth mesiodistal models published elsewhere (95–99). Overall, our model (Paper 1) performed better than all available strategies and can be used as a forensic tool for sex estimation in Portuguese samples (95–99). These results confirm the sexual dimorphism in the mesiodistal width of canines, premolars and molars in the upper and lower arches, and are of prospective importance, since no full-mouth models have been developed for the Portuguese population, only the canine index (100,101) or other anterior teeth-based indexes (99).

In general, several teeth are sexually dimorphic, and the average crown mesiodistal dimensions are larger in males than females. The results of this study (Paper1) confirm what has been shown previously, that canines are the most dimorphic teeth (97–99,102–104), but molars also show significant differences between the sexes (95,96,105,106). Among the elements included in our sex prediction model, the upper left canine, the lower right lateral incisor and the lower right canine were the most appropriate and had better reproducibility. With regard to performance, our developed model outperformed previously published indices with respect to AUC. Our model also outperformed other models (75.0% and 77.2% for accuracy and precision, respectively) in terms of accuracy and precision values. Moreover, in terms of sensitivity and specificity, this newly developed model presented the best combination of outcomes, outperforming Peckmann et al. (2016) (98) (model 4) for sensitivity and Acharya et al. (2007) (95) (model 2) for specificity.

One possible limitation of this study (Paper1) is the omission of maxillary teeth, mainly because the data from this analysis were derived from an orthodontic population whose primary objective was to study the mesiodistal proportion. However, second molars, along with first molars and premolars, have been found to be among the most missing teeth in two previous large studies of this population (107,108). It is interesting to note that none of these teeth were included in the final model. The fact that the new model was developed from the same sample studied, which may have influenced the results, is another possible limitation. In a future study, the new model should be further investigated using a new sample.

Differentiating minors from adults is important to prevent legal wrongdoing, especially in cases where valid identification documents are lacking to prevent legal wrongdoing (109,110). Nevertheless, because physical appearance and characteristics are not clearly associated with adulthood, age estimation is challenging, especially in distinguishing adolescents from young adults (ages 15 to early 20s) (109,111). From 15 years old, only the third molar teeth are immature, as this clinical condition is useful in forensic science (112).

In the Portuguese context, previous studies reported methods for estimating age based on dental measurements (113–117). Caldas et al (2011) (114) evaluated third molars. However, the method and the age range of these participants was not comparable. Therefore, to the best of our knowledge, this is the first study to validate the 0.08 I3M threshold in the Portuguese population (Paper2). Sensitivity indicates the ability of the I3M to correctly identify individuals who are 18 years of age or older (I3M<0.08). The specificity, on the other hand, is the ability to discriminate individuals who are younger than 18 years of age (I3M ≥ 0.08). Due to the higher specificity of this tool, Santiago et al. (2018) (28) reported that I3M is superior in individuals younger than 18 years old. This is of great importance in forensic science, as the erroneous processing of a minor as an adult would be a violation of his or her rights (28). The effect of age on the performance of the I3M is proposed to be based on the development of maturation according to sex, as females tend to develop at a younger age than males (118). This difference in maturation has been suggested to explain the discrepancies in I3M scores between the two sexes. However, further studies are required to fully understand the biological reasons for this issue.

Sex and age prediction models in forensic dentistry for the Portuguese population have been moderately studied, and there is still room for more tools and models to help forensic experts in mass disasters, such as the bridge collapse in Entre-os-Rios, in 2001 or the massive fires of Pedrogão Grande, in 2017.

Starting with methods based on dental measurements, Pereira et al. (2010) (99) analyzed and measured 80 dental casts using a digital caliper, and different odontometric indexes were defined using upper incisors and canines. Twelve different tooth measurements were recorded: the mesiodistal distance of each tooth, and the buccolingual and mesiobuccal-distolingual distance of the canines. From these six indices, the incisor index, the canine crown module and the canine robustness value were calculated. Based on these data, a discriminant function was created using the canine measurements to define the cut-off value between the sexes. They concluded that all measurements and indexes, except the incisor index, showed sexual dimorphism, similar to our results, and they also agreed that the canines are the most dimorphic teeth (Paper1).

The mandibular canine index has also been studied in the Portuguese population (100,101). It is based on mesiodistal measurements of both lower canines together with the measurement between the cuspids of the lower canines, in dental casts. The results of the mandibular canine index confirm the conclusion of our study that the canines are the most dimorphic teeth (Paper1). Both authors agree that the mandibular canine index is a good forensic tool; however, it should not be used in isolation but rather in combination with other predictive models to avoid possible procedural errors (100,101).

In 2013, Pereira et al. (119) compared radiographic methods with direct methods in an ex vivo specimen and, using different methods that included the complete measurement of the root or the width of the root, performed some ratios with these measurements. They concluded that radiographic methods are as accurate as the direct methods used in this study, with the advantage of being non-destructive. However, they pointed out that these methods should not be used in isolation but should be combined with other methods, as in other studies (100,101). Nevertheless, they did not consider direct (in the cadaver) or indirect (dental casts) non-destructive dental measurements as alternatives to radiographic methods.

In terms of radiographic methods, the most widely studied in the Portuguese population is the Demirjian method (113,114,120–122). Similar to other studies based on different subpopulations worldwide (66–68,123), this method tends to overestimate the age of Portuguese subjects (113,121,122), being more accurate up to 10 years of age, decreasing progressively up to 18 years of age and becoming insignificant beyond that age (113), as the original method only assesses up to the second molar. Caldas et al. (2011) (114) applied the same methodology to the 3rd molar and concluded that the methodology is geographically sensitive, as previously reported, but to a lesser extent (72,73). On the other hand, in the Portuguese population (114), Stage D can be used to predict an age of 16 years or older with an accuracy of 96.3% for females and 98.4% for males.

Other radiographic methods include Nolla (113,120), London atlas (122), Moorrees (120) and Cameriere's method (115,120–122,124).

Nolla's method tends to underestimate age, with a slightly higher accuracy compared to Demirjian, as does Moorrees, although they show worse stability results in terms of reliability in different age groups, making them more unpredictable and reducing their potential power as a forensic tool (113,120). The London Atlas shows good results, although in the Portuguese population it has only been studied in conjunction with the Demirjian and Cameriere methods, all of which show values close to 90% accuracy (122).

The Cameriere method has many advantages over the previous methods. It has already been validated worldwide and its application is simple, reproducible and non-destructive (Paper3). In the Portuguese population, it has already been validated for thresholds of 18 years (Paper3) as well as Pereira et al. (2021) (120), 16 years (120), 14 years (120,124) and 12 years (121,124), due to the different cut-offs defined, with accuracy values over 90%. Cameriere's method is well validated and justified in the Portuguese population, making it a reliable forensic tool.

In 2007, Cameriere (125) presented a new methodology that relates the pulp volume to the tooth volume, based on an osteological collection from Italy and validated in 2009 by another osteological collection from Coimbra, Portugal (116) and in 2013 by a study on recent cadavers (119). However, two more recent studies based on contemporary populations concluded that this pulp/tooth ratio does not allow dental identification in the Portuguese population, either in orthopantographies (115) or using CBCT (126). Both studies suggest further research with larger and more differentiated databases to verify the viability of the method (115,126).

It is important to note that these predictive models and techniques are constantly evolving and validated by research and studies. They play a crucial role in forensic dentistry in the accurate identification of individuals in the Portuguese population.

Evidence for sex determination in forensic dentistry was clearly summarized in our review (Paper3). Current knowledge is supported by evidence-based studies of low confidence, as the methodological quality of the included SRs ranged from critically low to low quality.

In addition to radiographic and non-radiographic dental techniques, our work (Paper3) enabled us to conclude that teeth, being the most resistant organs in the human body, remain intact for a significant period of time, making them a prime source for DNA collection, which is the most reliable source for sex determination, and that age does not seem to influence sex determination by DNA analysis (127). Using DNA amplification techniques such as polymerase chain reaction (PCR), DNA can be successfully collected and processed for sex determination (127). The correct interpretation of the results is hampered by the lack of standardization of DNA sources and conditions (127).

Furthermore, AI models related to sex determination have only been applied to the identification of sexual dimorphism in canines by means of orthopantomography. Two standard methods of sex determination, discriminant analysis and logistic regression, were outperformed by a multi-layer perceptron and an artificial neural network (42).

The evidence for age estimation in forensic dentistry was clearly summarized in our review (Paper4). Current knowledge is supported by evidence-based studies of low to moderate confidence, at best ranging from critically low to high quality.

Most studies are based on methods developed from two-dimensional radiographs, such as the methods of Demirjian and Willems, which have been validated worldwide (66–68). Both methods tend to overestimate age (66–68). Recently, several studies have investigated whether 3D imaging can be applied in forensic dentistry (63,64). AI technology has already been tested for age estimation and appears to be a potential forensic tool (42).

Santiago et al. (28) concluded that regardless of the population studied, I3M has a high accuracy in discriminating whether an individual has reached 18 years of age and has been validated worldwide in several subpopulations. In terms of sex, a trend of better results was observed in males, but high values of accuracy, sensitivity, and specificity were also achieved in females (28).

The pulp/tooth volume ratio can be analyzed using CBCT and CT scan reconstructions. The volume ratio may be an interesting tool to predict the age after the third molars have matured roots, around the second decade of life (63). MRI, which uses strong magnetic fields and radio waves to generate images, has emerged as a viable alternative due to the ethical implications of using ionized radiation for nondiagnostic indications (64).

To overcome the subjectivity of examiners, AI-based automated systems were developed. The AI model that has performed the best has been the deep learning convolutional neural network approach, with similar levels of accuracy when compared to trained researchers. However, AI-based models have not yet provide sufficient field validation for routine use (42).

Reporting biomedical research clearly, accurately, and transparently remains an important issue. To address the second goal of our project, we categorized and ranked the quality of scientific evidence in forensic dentistry based on the degree to which the evidence is free of bias and the ability to transparently report all steps from collecting data to reaching conclusions. Randomized controlled trials are considered the "gold standard" and systematic reviews with meta-analysis of randomized controlled trials are considered the highest level of evidence (128).

To make a systematic review valuable to users, authors should provide a transparent, complete, and accurate account of why the review was done, what was done (such as study identification and selection), and what was found (such as study characteristics and meta-analysis results). Up-to-date reporting guidelines make it easier for authors to achieve this goal (129,130).

The quality of the peer review report and the manuscript under review are likely to be improved by peer review supplemented by the use of reporting guidelines. There is little information available to readers regarding the quality of what is published in journals. Journals should provide this information more proactively, including through regular audits and feedback (129,131,132). An audit of the quality of the reports published by a journal could provide important information for authors, editors, peer reviewers and readers to identify problems and opportunities to improve the quality of the published articles. The publication of such information would send a strong positive signal about the journal's commitment to openness, data sharing, and continuous quality improvement (129,131).

Currently, several reporting guidelines are available to assist authors, editors, and peer reviewers, such as TRIPOD, PRISMA or SWiM (82,90,133). However, improvements have been slow to materialize (130). While other tools are integrated into journal editorial management processes, electronic algorithms are being developed to facilitate the selection of the correct reporting guidelines. If journals were to embrace the auditing of the quality of the articles they publish, it would provide them and their readers with important feedback for the improvement of their product (129,130).

The publication of systematic reviews in the field of dentistry has been rapid, and as a result, there has been an increase in the number of reviews of systematic reviews. These reviews synthesize the results of the included systematic reviews to provide a more comprehensive view of a given clinical question (93) and are called umbrella reviews, reviews of reviews, or overviews of reviews. Often, using established tools such as AMSTAR, the authors of these umbrella reviews also assess and report on the quality of the included systematic reviews (82). This presents an unprecedented opportunity to disaggregate the overall confidence data from comprehensive reviews to draw conclusions about the quality of reporting of systematic reviews on an even broader scale (83,84).

Umbrella reviews have the advantage of providing a comprehensive review of the available systematic reviews, using a transparent methodology. However, one

limitation must be considered when interpreting the results. The individual studies included were not examined in any of the systematic reviews and the conclusions are, therefore, based on the interpretation of the authors.

5.2. Concluding Remarks

Paper I – Sex estimation is a vital step in Forensic Medicine for body identification in both single and mass disaster events. In the Portuguese scenario, sex estimation models using dental hard tissues are scarce and previous proposed models lack consistency and only accounted for a small subset of teeth. Overall, our model outperformed all available strategies and might be used as a forensic tool for sex estimation in Portuguese samples.

Considering the limitations of this study, it was found that there is a prevalence of sexual dimorphism in all teeth except the incisors, and the canines exhibited the most noticeable difference between sexes, followed by the first mandibular molars and premolars.

Through a stepwise adjusted logistic regression procedure, a suitable model for sex determination was developed. The reduced model was based on the upper left canine (2.3), the lower right lateral incisor (4.2) and the lower right canine (4.3), achieving an accuracy of 75%.

Paper II – The present study is the first to test the validity of I3M in a Portuguese sample. The estimated age of majority was correlated with the chronological age, therefore, I3M is a suitable method in legal and forensic purposes to identify minors from adults in the Portuguese population. Furthermore, the cut-off value used (0.08) is predictable and useful for discriminating individuals younger than 18 years of age (high specificity in both sexes).

The overall accuracy, sensitivity and specificity were 92.8%, 90.7% and 94.9%, respectively. The I3M values decreased as age increased across all age groups in both sexes, showing that the lower third molar mineralization occurred earlier in females than in males, Moreover, I3M was more accurate in male participants.

Paper III – In Forensic Dentistry, sex determination is a key element during the process of human identification. This process is facilitated because the pool of missing people would be half, which allows to speed up the matching operation. Umbrella reviews benefit from its comprehensive review of the available systematic reviews, using a transparent methodology. However, one limitation must be considered when interpreting the results. In each systematic review, the individual studies included were not explored. Thus, the conclusions of this Umbrella review are based on the interpretations of the authors. This umbrella review reports the most common mistakes performed in SRs and will pave the way for more robust evidence-based research in the future.
The methodological quality of the included SRs ranged from critically low to low quality, and therefore, the current knowledge is supported by low-confidence evidence-based studies. Although DNA is the best source for sex determination, forensic dental methods for sex estimation are somewhat reliable.

Paper IV – Overall, these results indicate that due to the poor quality of many of the studies cited here, some of the forensic tools used today may be outdated or misused, and some results must be analyzed with caution. Current evidence regarding forensic dental methods for estimating age is supported by systematic reviews with low to moderate confidence. The Willems method is more accurate and less prone to overestimation. Although some authors attribute this heterogeneity to methodological errors, most methods appear to be geographically sensitive. Cameriere's index has high accuracy, regardless of the population studied. 3D imaging is an AI technology that, although on the rise, still lacks validation in the field. This comprehensive review reports the most common errors made in SRs and will pave the way for more robust, evidence-based research in the future.

Overall, with this Thesis, we concluded that:

- Current evidence on Forensic Dentistry methods for sex determination and age estimation is supported by critically low to low evidence and low to moderate evidence, respectively.

- All human teeth exhibit a small degree of sexual dimorphism, except for the incisors, with the canines and first molars the most dimorphic teeth.
- Although DNA is the best source for sex determination, forensic dental methods for sex estimation are somewhat reliable.
- Most forensic methods seem to be geographically sensitive.
- Willems' method is more accurate and less prone to overestimation.
- I3M is a suitable method for legal and forensic purposes to identify minors from adults in the Portuguese population of both sexes.
- Following structured guidelines, such as TRIPOD, PRISMA or SWiM, lead to more transparent publications and better evidence quality.

5.3. Future Directions

Future directions in forensic dentistry estimation methods include the need for less invasive techniques and a combination of different measurement techniques to improve accuracy. There is also a need for research on populations of African and Asian descent, as current studies in these areas are lacking. Additionally, potential future developments aim to simplify and reduce the subjectivity of the analysis process. These developments could help improve the overall effectiveness of forensic dentistry in legal investigations through scientific validation of AI tools and support.

AI technologies can be used to collect, process, and organize patient-related data sets for individualized dental treatment and have the potential to be used for automated diagnostics, predictive measures, and classification or identification tools in forensic dentistry. However, challenges and limitations, such as the lack of standardized reporting formats and the size of data sets, require further development before AI can be implemented in routine clinical practice.

Despite the number of scientific publications in forensic dentistry, its quality is still quite poor. The dissemination of well-structured and scientifically validated guidelines allows for greater transparency, and consequently, better quality of evidence.

Future directions regarding the quality of dental research may include the development of more comprehensive and rigorous tools that assess other

aspects of research quality, including the research question, methods, analysis, and interpretation of results, making quality assessment more transparent and accessible to researchers, clinicians, and patients. This will help ensure that only high-quality research is used to inform decisions regarding clinical practice and public health policy.

In addition to these specific directions, there is also a need for a more general shift in the way that the quality of dental research is assessed. Rather than focusing on identifying individual studies of poor quality, the goal should be to develop a culture of continuous quality improvement in dental research. This will require researchers, clinicians, and patients to work together to identify and address the challenges that impede the production of high-quality dental research and to create a culture of continuous quality improvement in dental research that will benefit all.

CHAPTER 6

6. References

1. Stavrianos C, Kokkas A, Eliades A, Andreopoul E. Applications of Forensic Dentistry: Part-II. Res J Med Sci [Internet]. 2010 Mar 1;4(3):187–94.
2. Pretty IA, Sweet D. A look at forensic dentistry--Part 1: The role of teeth in the determination of human identity. Br Dent J [Internet]. 2001 Apr 14;190(7):359–66.
3. Gupta S, Agnihotri A, Chandra A, Gupta OP. Contemporary practice in forensic odontology. Vol. 18, Journal of Oral and Maxillofacial Pathology. Wolters Kluwer Medknow Publications; 2014. p. 244–50.
4. Prajapati G, Sarode SC, Sarode GS, Shelke P, Awan KH, Patil S. Role of forensic odontology in the identification of victims of major mass disasters across the world: A systematic review. Vol. 13, PLoS ONE. Public Library of Science; 2018.
5. Corte-Real A, Vieira DN. Identificação em Medicina Dentária Forense. 1st Edition. Vol. 1st Chapter. Imprensa da Universidade de Coimbra; 2015. 15–32 p.
6. Senn D, Weems R. Manual of Forensic Odontology. 5th Edition. Senn D, Weems R, editors. Vol. Chapter 1. 2021. 1–39 p.
7. Balachander N, Babu NA, Jimson S, Priyadharsini C, Masthan KMK. Evolution of forensic odontology: An overview. Vol. 7, Journal of Pharmacy and Bioallied Sciences. Wolters Kluwer Medknow Publications; 2015. p. S176–80.
8. Nola MF. Paul revere and forensic dentistry. Vol. 181, Military Medicine. Association of Military Surgeons of the US; 2016. p. 714–5.
9. Singh K, Bhullar RPK, Agrawal A, Chaudhary H, Anandani C, Thakral A. Teeth and their secrets - Forensic dentistry. Medico-Legal Update. 2012;12(2):85–9.
10. Devadiga A. What′s the deal with dental records for practicing dentists? Importance in general and forensic dentistry. J Forensic Dent Sci. 2014;6(1):9.
11. Trengrove HG, Gray A. The role of military dental capabilities in mass fatality situations. Mil Med. 2013 May;178(5):523–8.
12. Anuja P, Doggalli N. Software in forensic odontology :P Anuja, Nagabhushana Doggalli, Indian Journal of Multidisciplinary Dentistry Anuja P, Doggalli N. Software in forensic odontology [Internet]. Vol. 8, Indian J Multidiscip Dent. 2018.
13. Gawali R. Forensic Odontology for General Dentists. Journal of Dental Research and Review. 2021;8(2):126–31.
14. Cameriere R, Ferrante L, De Angelis D, Scarpino F, Galli F. The comparison between measurement of open apices of third molars and Demirjian stages to test chronological age of over 18 year olds in living subjects. Int J Legal Med. 2008 Nov;122(6):493–7.
15. Interpol. Disaster Victim Identification Guide (DVI). International Criminal Police Organization. 2018;
16. Zorba E, Moraitis K, Manolis SK. Sexual dimorphism in permanent teeth of modern Greeks. Forensic Sci Int. 2011 Jul 15;210(1–3):74–81.
17. Bérgamo A, Alves da Silva R. Dental Age Estimation Methods in Forensic Dentistry: Literature Review. Forensic Science Today. 2016 Feb 5;004–9.
18. Cattaneo C. Forensic anthropology: developments of a classical discipline in the new millennium. Forensic Sci Int. 2007 Jan 17;165(2–3):185–93.
19. Campobasso C Pietro, Introna F. The forensic entomologist in the context of the forensic pathologist's role. Forensic Sci Int [Internet]. 2001 Aug;120(1–2):132–9.
20. Işcan MY, Kedici PS. Sexual variation in bucco-lingual dimensions in Turkish dentition. Forensic Sci Int. 2003 Nov 26;137(2–3):160–4.
21. Mitsea AG, Moraitis K, Leon G, Nicopoulou-Karayianni K, Spiliopoulou C. Sex determination by tooth size in a sample of Greek population. HOMO- Journal of Comparative Human Biology. 2014;65(4):322–9.
22. Peckmann TR, Logar C, Garrido-Varas CE, Meek S, Pinto XT. Sex determination using the mesio-distal dimension of permanent maxillary incisors and canines in a modern Chilean population. Science and Justice. 2016 Mar 1;56(2):84–9.
23. Sweet D. Forensic dental identification. Forensic Sci Int. 2010 Sep 1;201(1–3):3–4.
24. Rathod V, Desai V, Pundir S, Dixit S, Chandraker R. Role of forensic dentistry for dental practitioners: A comprehensive study. J Forensic Dent Sci. 2017;9(2):108.
25. Silva RF, Franco A, Dias PEM, Gonçalves AS, Paranhos LR. Interrelationship between forensic radiology and forensic odontology - A case report of identified skeletal remains. Journal of Forensic Radiology and Imaging. 2013 Oct;1(4):201–6.
26. Brough AL, Morgan B, Rutty GN. The basics of disaster victim identification. Vol. 3, Journal of Forensic Radiology and Imaging. Elsevier Ltd; 2015. p. 29–37.
27. Anastácio AC, Serras C, Vargas de Sousa Santos RF, Palmela Pereira C. Validation of Cameriere's medical-legal age estimation method using seconds premolars in a Portuguese population. J Forensic Leg Med. 2018 Nov 1;60:30–4.
28. Santiago BM, Almeida L, Cavalcanti YW, Magno MB, Maia LC. Accuracy of the third molar maturity index in assessing the legal age of 18 years: a systematic review and meta-analysis. Int J Legal Med. 2018 Jul 1;132(4):1167–84.
29. Capitaneanu C, Willems G, Thevissen P. A systematic review of odontological sex estimation methods. J Forensic Odontostomatol [Internet]. 2017 Dec 1;35(2):1–19.
30. Mohammed F, Fairozekhan AT, Bhat S, Menezes RG. Forensic Odontology [Internet]. StatPearls. 2019.

31. Waleed P, Baba F, Alsulami S, Tarakji B. Importance of dental records in forensic dental identification. Acta Informatica Medica. 2015;23(1):49–52.
32. Krishan K, Kanchan T, Garg AK. Dental Evidence in Forensic Identification-An Overview, Methodology and Present Status. Vol. 9, The Open Dentistry Journal. 2015.
33. Hinchliffe J. Forensic odontology, part 1. Dental identification. Vol. 210, British Dental Journal. 2011. p. 219–24.
34. Manjunath K, Sivapathasundharam B, Saraswathi TR. Analysis of Enamel Rod End Patterns on Tooth Surface for Personal Identification-Ameloglyphics. J Forensic Sci. 2012 May;57(3):789–93.
35. Sabarigirinathan C, Vinayagavel K, Meenakshi A, Selvamani C, Sriramaprabu G, Sivasakthikumar S, et al. Palatal Rugae in Forensic Odontology-A Review. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN [Internet]. 2015;14(10):83–7.
36. Caldas IM, Magalhães T, Afonso A. Establishing identity using cheiloscopy and palatoscopy. Vol. 165, Forensic Science International. Elsevier Ireland Ltd; 2007. p. 1–9.
37. Ata-Ali J, Ata-Ali F. Forensic dentistry in human identification: A review of the literature. J Clin Exp Dent. 2014;6(2).
38. Khamis MF, Taylor JA, Malik SN, Townsend GC. Odontometric sex variation in Malaysians with application to sex prediction. Forensic Sci Int. 2014;234:183.e1-183.e7.
39. Silva AM, Pereira ML, Gouveia S, Tavares JN, Azevedo Á, Caldas IM. A new approach to sex estimation using the mandibular canine index. Med Sci Law. 2016 Jan 1;56(1):7–12.
40. Prabhu S, Acharya AB. Odontometric sex assessment in Indians. Forensic Sci Int. 2009 Nov 20;192(1–3):129.e1-129.e5.
41. Pereira C, Bernardo M, Pestana D, Santos JC, Mendonça MC de. Contribution of teeth in human forensic identification - Discriminant function sexing odontometrical techniques in Portuguese population. J Forensic Leg Med. 2010 Feb;17(2):105–10.
42. Khanagar SB, Vishwanathaiah S, Naik S, A. Al-Kheraif A, Devang Divakar D, Sarode SC, et al. Application and performance of artificial intelligence technology in forensic odontology – A systematic review. Vol. 48, Legal Medicine. Elsevier B.V.; 2021.
43. Maulani C, Auerkari EI. Molecular analysis for sex determination in forensic dentistry: a systematic review. Vol. 10, Egyptian Journal of Forensic Sciences. Springer Science and Business Media Deutschland GmbH; 2020.
44. Cameriere R, Pacifici A, Viva S, Carbone D, Pacifici L, Polimeni A. Adult or not? Accuracy of Cameriere's cut-off value for third molar in assessing 18 years of age for legal purposes. Minerva Stomatol [Internet]. 2014 Sep;63(9):283–94.
45. Angadi P V., Hemani S, Prabhu S, Acharya AB. Analyses of odontometric sexual dimorphism and sex assessment accuracy on a large sample. J Forensic Leg Med. 2013;20(6):673–7.
46. Jeon HM, Jang SM, Kim KH, Heo JY, Ok SM, Jeong SH, et al. Dental Age Estimation in Adults: A Review of the Commonly Used Radiological Methods. J Oral Med Pain. 2014 Dec 30;39(4):119–26.
47. Willems G. A review of the most commonly used dental age estimation techniques. J Forensic Odontostomatol [Internet]. 2001 Jun;19(1):9–17.
48. Cunha E, Baccino E, Martrille L, Ramsthaler F, Prieto J, Schuliar Y, et al. The problem of aging human remains and living individuals: A review. Vol. 193, Forensic Science International. Elsevier Ireland Ltd; 2009. p. 1–13.
49. da Silva PR, Lopes MC, Martins-Filho IE, Haye Biazevic MG, Michel-Crosato E. Tooth crown mesiodistal measurements for the determination of sexual dimorphism across a range of populations: A systematic review and meta-analysis. J Forensic Odontostomatol. 2019 May 1;37(1):2–19.
50. Pratapiene M, CICCIù M, Juodzbalys G. Canines mesiodistal measures as the key to sex prediction: a systematic review and meta-analysis. Minerva Pediatr. 2016 Aug;68(4):288–98.
51. Rao NG, Rao NN, Pai ML, Kotian MS. Mandibular canine index-a clue for establishing sex identity. Forensic Sci Int [Internet]. 1989 Aug;42(3):249–54.
52. Kapila R, Nagesh • K S, Iyengar AR, Mehkri S. Sexual Dimorphism in Human Mandibular Canines: A Radiomorphometric Study in South Indian Population [Internet]. Vol. 5, Dental Clinics, Dental Prospects Original Article J Dent Res Dent Clin Dent Prospects. 2011.
53. Boaz K, Gupta C. Dimorphism in human maxillary and madibular canines in establishment of gender. J Forensic Dent Sci. 2009;1(1):42.
54. Joseph AP, Rajeesh Mohammed PK, Kumar VR. How reliable is sex differentiation from teeth measurements. Oral & Maxillofacial Pathology Journal. 2013;4(1):289–92.
55. Acharya AB, Mainali S. Univariate sex dimorphism in the Nepalese dentition and the use of discriminant functions in gender assessment. Forensic Sci Int. 2007 Nov 15;173(1):47–56.
56. Acharya AB, Prabhu S, Muddapur M V. Odontometric sex assessment from logistic regression analysis. Int J Legal Med. 2011 Mar;125(2):199–204.
57. Maber M, Liversidge HM, Hector MP. Accuracy of age estimation of radiographic methods using developing teeth. Forensic Sci Int. 2006 May 15;159(1).
58. Mani SA, Naing L, John J, Samsudin AR. Comparison of two methods of dental age estimation in 7-15-year-old Malays. Int J Paediatr Dent. 2008 Sep;18(5):380–8.

59. Franco A, de Oliveira MN, Vidigal MTC, Blumenberg C, Pinheiro AA, Paranhos LR. assessment of dental age estimation methods applied to Brazilian children: A systematic review and meta-analysis. Vol. 50, Dentomaxillofacial Radiology. British Institute of Radiology; 2021.
60. Hostiuc S, Diaconescu I, Rusu MC, Negoi I. Age estimation using the cameriere methods of open apices: A meta-analysis. Healthcare (Switzerland). 2021;9(2).
61. Marroquin TY, Karkhanis S, Kvaal SI, Vasudavan S, Kruger E. Age estimation in adults by dental imaging assessment systematic review. Forensic Sci Int. 2017 Jun 1;275:203–11.
62. Diaconescu I, Isailă OM, Hostiuc S. Accuracy of the Chaillet's Method for Assessing the Age in Subadults. A Meta-Analysis. Curr Health Sci J [Internet]. 2021;47(2):196–203.
63. Bjørk MB, Kvaal SI. CT and MR imaging used in age estimation: a systematic review. J Forensic Odontostomatol [Internet]. 2018 May 30;36(1):14–25.
64. De Tobel J, Bauwens J, Parmentier GIL, Franco A, Pauwels NS, Verstraete KL, et al. Magnetic resonance imaging for forensic age estimation in living children and young adults: a systematic review. Vol. 50, Pediatric Radiology. Springer Science and Business Media Deutschland GmbH; 2020. p. 1691–708.
65. Demirjian A, Goldstein H, Tanner JM. A new system of dental age assessment. Hum Biol [Internet]. 1973 May;45(2):211–27.
66. Esan TA, Yengopal V. The Demirjian versus the Willems method for dental age estimation in different populations: A meta-analysis of published studies. PLoS One. 2017 Nov 1;12(11).
67. Hostiuc S, Edison SE, Diaconescu I, Isaila OM. Accuracy of the Demirjian's method for assessing the age in children, from 1973 to 2020. A meta-analysis. Leg Med. 2021 Sep 1;52.
68. Jayaraman J, Wong HM, King NM, Roberts GJ. The French-Canadian data set of Demirjian for dental age estimation: A systematic review and meta-analysis. Vol. 20, Journal of Forensic and Legal Medicine. 2013. p. 373–81.
69. Prasad H, Kala N. Accuracy of two dental age estimation methods in the Indian population - A meta-analysis of published studies. J Forensic Odontostomatol. 2019 Dec 30;37(3):2–11.
70. Yan J, Lou X, Wang Y. Assessment of dental age of children aged 3.5 to 16.9 years using Demirjian's method: A meta-analysis based on 26 studies. PLoS One. 2013 Dec 18;8(12).
71. Mincer HH, Harris EF, Berryman HE, ABFO Study T. The A.B.F.O. Study of Third Molar Development and Its Use As an Estimator of Chronological Age [Internet]. Vol. 38, Journal of Forensic Sciences, JFSCA. 1993.
72. Haglund M, Mörnstad H. A systematic review and meta-analysis of the fully formed wisdom tooth as a radiological marker of adulthood. Int J Legal Med. 2019 Jan 14;133(1):231–9.
73. Rolseth V, Mosdøl A, Dahlberg PS, Ding Y, Bleka Ø, Skjerven-Martinsen M, et al. Age assessment by Demirjian's development stages of the third molar: a systematic review. Vol. 29, European Radiology. Springer Verlag; 2019. p. 2311–21.
74. Mohd Yusof MYP, Wan Mokhtar I, Rajasekharan S, Overholser R, Martens L. Performance of Willem's dental age estimation method in children: A systematic review and meta-analysis. Forensic Sci Int. 2017 Nov 1;280:245.e1-245.e10.
75. Sehrawat JS, Singh M. Willems method of dental age estimation in children: A systematic review and meta-analysis. Vol. 52, Journal of Forensic and Legal Medicine. Churchill Livingstone; 2017. p. 122–9.
76. Wang J, Ji F, Zhai Y, Park H, Tao J. Is Willems method universal for age estimation: A systematic review and meta-analysis. Vol. 52, Journal of Forensic and Legal Medicine. Churchill Livingstone; 2017. p. 130–6.
77. Cameriere R, Ferrante L, Cingolani M. Age estimation in children by measurement of open apices in teeth. Int J Legal Med. 2006 Jan;120(1):49–53.
78. Vandevoort FM, Bergmans L, Van Cleynenbreugel J, Bielen DJ, Lambrechts P, Wevers M, et al. Age calculation using X-ray microfocus computed tomographical scanning of teeth: a pilot study. J Forensic Sci [Internet]. 2004 Jul;49(4):787–90.
79. Baumann P, Widek T, Merkens H, Boldt J, Petrovic A, Urschler M, et al. Dental age estimation of living persons: Comparison of MRI with OPG. Forensic Sci Int. 2015 Aug 1;253:76–80.
80. Fusar-Poli P, Radua J. Ten simple rules for conducting umbrella reviews. Evid Based Ment Health. 2018 Aug 1;21(3):95–100.
81. Moher D, Liberati A, Tetzlaff J, Altman DG, Antes G, Atkins D, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. Vol. 6, PLoS Medicine. Public Library of Science; 2009.
82. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. J Clin Epidemiol. 2021 Jun 1;134:178–89.
83. Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, et al. AMSTAR 2: A critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ (Online). 2017;358.
84. Elangovan S, Kumar S. Systematic reviews: Do they deserve our blind trust? Journal of the American Dental Association. American Dental Association; 2023.

85. Shea BJ, Hamel C, Wells GA, Bouter LM, Kristjansson E, Grimshaw J, et al. AMSTAR is a reliable and valid measurement tool to assess the methodological quality of systematic reviews. J Clin Epidemiol. 2009 Oct;62(10):1013–20.
86. Shi X, Wallach JD. Umbrella reviews: a useful study design in need of standardization. The BMJ. BMJ Publishing Group; 2022.
87. Rosenthal R. Writing Meta-Analytic Reviews. Vol. 118, Psychological Bulletin. Hunter & Schmidt; 1995.
88. Olkin I. Statistical and theoretical considerations in meta-analysis. J Clin Epidemiol [Internet]. 1995 Jan;48(1):133–46; discussion 147.
89. Liberati A, Altman DG, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. In: Journal of clinical epidemiology. 2009. p. e1–34.
90. Campbell M, McKenzie JE, Katikireddi SV, Brennan SE, Ellis S, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: Reporting guideline. The BMJ. 2020 Jan 16;368.
91. Cumpston MS, Brennan SE, Ryan R, McKenzie JE. Synthesis methods other than meta-analysis were commonly used but seldom specified: survey of systematic reviews. J Clin Epidemiol. 2023 Apr 1;156:42–52.
92. Burda BU, Norris SL, Holmer HK, Ogden LA, Smith MEB. Quality varies across clinical practice guidelines for mammography screening in women aged 40-49 years as assessed by AGREE and AMSTAR instruments. J Clin Epidemiol. 2011;64(9):968–76.
93. Elangovan S, Kumar S. Systematic reviews: Do they deserve our blind trust? Journal of the American Dental Association. American Dental Association; 2023.
94. Jayaraman J, Nagendrababu V, Pulikkotil SJ, Innes NP. Critical appraisal of methodological quality of Systematic Reviews and Meta-analysis in Paediatric Dentistry journals. Vol. 28, International Journal of Paediatric Dentistry. Blackwell Publishing Ltd; 2018. p. 548–60.
95. Acharya AB, Mainali S. Univariate sex dimorphism in the Nepalese dentition and the use of discriminant functions in gender assessment. Forensic Sci Int. 2007 Nov 15;173(1):47–56.
96. Angadi P V., Hemani S, Prabhu S, Acharya AB. Analyses of odontometric sexual dimorphism and sex assessment accuracy on a large sample. J Forensic Leg Med. 2013;20(6):673–7.
97. Mitsea AG, Moraitis K, Leon G, Nicopoulou-Karayianni K, Spiliopoulou C. Sex determination by tooth size in a sample of Greek population. HOMO- Journal of Comparative Human Biology. 2014;65(4):322–9.
98. Peckmann TR, Logar C, Garrido-Varas CE, Meek S, Pinto XT. Sex determination using the mesio-distal dimension of permanent maxillary incisors and canines in a modern Chilean population. Science and Justice. 2016 Mar 1;56(2):84–9.
99. Pereira C, Bernardo M, Pestana D, Santos JC, Mendonça MC de. Contribution of teeth in human forensic identification - Discriminant function sexing odontometrical techniques in Portuguese population. J Forensic Leg Med. 2010 Feb;17(2):105–10.
100. Azevedo Á, Pereira ML, Gouveia S, Tavares JN, Caldas IM. Sex estimation using the mandibular canine index components. Forensic Sci Med Pathol. 2019 Jun 14;15(2):191–7.
101. Silva AM, Pereira ML, Gouveia S, Tavares JN, Azevedo Á, Caldas IM. A new approach to sex estimation using the mandibular canine index. Med Sci Law. 2016 Jan 1;56(1):7–12.
102. Khamis MF, Taylor JA, Malik SN, Townsend GC. Odontometric sex variation in Malaysians with application to sex prediction. Forensic Sci Int. 2014;234:183.e1-183.e7.
103. Phulari RashmiGS, Rathore R, Talegaon T, Jariwala P. Comparative assessment of maxillary canine index and maxillary first molar dimensions for sex determination in forensic odontology. J Forensic Dent Sci. 2017;9(2):110.
104. Acharya AB, Prabhu S, Muddapur M V. Odontometric sex assessment from logistic regression analysis. Int J Legal Med. 2011 Mar;125(2):199–204.
105. Viciano J, Lázaro S, Alemán I. Sex estimation based on deciduous and permanent dentition in a contemporary spanish population. Am J Phys Anthropol. 2013 Sep;152(1):31–43.
106. Martins Filho IE, Capp TT, Biazevic MGH, Crosato E. Sexual dimorphism using odontometric indexes: Analysis of three statistical techniques. J Forensic Leg Med. 2016 Nov 1;44:37–42.
107. Botelho J, Machado V, Proença L, Alves R, Cavacas MA, Amaro L, et al. Study of Periodontal Health in Almada-Seixal (SoPHiAS): a cross-sectional study in the Lisbon Metropolitan Area. Sci Rep. 2019 Dec 1;9(1).
108. Machado V, Botelho J, Amaral A, Proença L, Alves R, Rua J, et al. Prevalence and extent of chronic periodontitis and its risk factors in a Portuguese subpopulation: A retrospective cross-sectional study and analysis of Clinical Attachment Loss. PeerJ. 2018;2018(7).
109. Cunha E, Baccino E, Martrille L, Ramsthaler F, Prieto J, Schuliar Y, et al. The problem of aging human remains and living individuals: A review. Vol. 193, Forensic Science International. Elsevier Ireland Ltd; 2009. p. 1–13.
110. Deitos AR, Costa C, Michel-Crosato E, Galić I, Cameriere R, Biazevic MGH. Age estimation among Brazilians: Younger or older than 18? J Forensic Leg Med. 2015 May 29;33:111–5.
111. Angelakopoulos N, De Luca S, Velandia Palacio LA, Coccia E, Ferrante L, Cameriere R. Third molar maturity index (I3M) for assessing age of majority: study of a black South African sample. Int J Legal Med. 2018 Sep 1;132(5):1457–64.

112. Galić I, Vodanović M, Janković S, Mihanović F, Nakaš E, Prohić S, et al. Dental age estimation on Bosnian-Herzegovinian children aged 6-14 years: Evaluation of Chaillet's international maturity standards. J Forensic Leg Med. 2013 Jan;20(1):40–5.
113. Tomás LF, Mónico LSM, Tomás I, Varela-Patiño P, Martin-Biedma B. The accuracy of estimating chronological age from Demirjian and Nolla methods in a Portuguese and Spanish sample. BMC Oral Health. 2014 Dec 23;14(1).
114. Caldas IM, Júlio P, Simões RJ, Matos E, Afonso A, Magalhães T. Chronological age estimation based on third molar development in a Portuguese population. Int J Legal Med. 2011 Mar;125(2):235–43.
115. Anastácio AC, Serras C, Vargas de Sousa Santos RF, Palmela Pereira C. Validation of Cameriere's medical-legal age estimation method using seconds premolars in a Portuguese population. J Forensic Leg Med. 2018 Nov 1;60:30–4.
116. Cameriere R, Cunha E, Sassaroli E, Nuzzolese E, Ferrante L. Age estimation by pulp/tooth area ratio in canines: Study of a Portuguese sample to test Cameriere's method. Forensic Sci Int. 2009 Dec 15;193(1–3):128.e1-128.e6.
117. Pavlović S, Palmela Pereira C, Vargas de Sousa Santos RF. Age estimation in Portuguese population: The application of the London atlas of tooth development and eruption. Forensic Sci Int. 2017 Mar 1;272:97–103.
118. Veldhuis JD, Roemmich JN, Rogol AD. Gender and Sexual Maturation-Dependent Contrasts in the Neuroregulation of Growth Hormone Secretion in Prepubertal and Late Adolescent Males and Females-A General Clinical Research Center-Based Study* [Internet]. Vol. 85, The Journal of Clinical Endocrinology & Metabolism Printed. 2000.
119. CP P, R C, D P. Legal Medical Age Estimation in Portuguese Adult Cadavers: Evaluation of the Accuracy of Forensic Dental Invasive and Non-Invasive Methods. J Forensic Sci Criminol. 2013 Oct;1(2).
120. Palmela Pereira C, Rodrigues A, Santos A, Salvado F, Santos R, Cameriere R. Cut-off for the legal ages in the Portuguese Population by Third Maturity Index: Measures of Accuracy. Arch Oral Biol. 2021 May 1;125.
121. Pereira CP, Belo C, Sardinha J, Santos R, Salvado F, Cameriere R. Quantitative and Qualitative Approaches for Dental Age Assessment in Sub Adult Portuguese Population: European Regression Formula and Demirjian Stages. Acta Stomatol Croat. 2022;56(3):288–98.
122. Pereira CP, Santos R, Nushi V, Lameiro MV, Antunes P, Carvalho R, et al. Dental age assessment: scoring systems and models from the past until the present—how is it presented in the court? Int J Legal Med. 2023 Sep 1;137(5):1497–504.
123. Franco A, de Oliveira MN, Vidigal MTC, Blumenberg C, Pinheiro AA, Paranhos LR. assessment of dental age estimation methods applied to Brazilian children: A systematic review and meta-analysis. Vol. 50, Dentomaxillofacial Radiology. British Institute of Radiology; 2021.
124. Augusto D, Pereira CP, Rodrigues A, Cameriere R, Salvado F, Santos R. Dental age assessment by I2M and I3M: Portuguese legal age thresholds of 12 and 14 year olds. Acta Stomatol Croat. 2021 Mar 1;55(1):45–55.
125. Cameriere R, Ferrante L, Belcastro MG, Bonfiglioli B, Rastelli E, Cingolani M. Age estimation by pulp/tooth ratio in canines by peri-apical X-rays. J Forensic Sci. 2007 Jan;52(1):166–70.
126. Pires AC, Vargas de Sousa Santos RF, Pereira CP. Dental age assessment by the pulp/tooth area proportion in cone beam computed tomography: is medico-legal application for age estimation reliable? J Forensic Odontostomatol [Internet]. 2021 Aug 30;39(2):2–14.
127. Maulani C, Auerkari EI. Molecular analysis for sex determination in forensic dentistry: a systematic review. Vol. 10, Egyptian Journal of Forensic Sciences. Springer Science and Business Media Deutschland GmbH; 2020.
128. Sutherland SE. Evidence-based dentistry: Part IV. Research design and levels of evidence. J Can Dent Assoc [Internet]. 2001;67(7):375–8.
129. Moher D. Reporting guidelines: Doing better for readers. Vol. 16, BMC Medicine. BioMed Central Ltd.; 2018.
130. Altman DG, Moher D. Declaration of transparency for each research article. Vol. 347, BMJ (Online). 2013.
131. Pauletto P, Polmann H, Réus JC, de Oliveira JMD, Chaves D, Lehmkuhl K, et al. Critical appraisal of systematic reviews of intervention in dentistry published between 2019-2020 using the AMSTAR 2 tool. Evid Based Dent [Internet]. 2022 Sep 14.
132. Faggion CM, Monje A, Wasiak J. Appraisal of systematic reviews on the management of peri-implant diseases with two methodological tools. J Clin Periodontol. 2018 Jun 1;45(6):754–66.
133. Moons KGM, Altman DG, Reitsma JB, Ioannidis JPA, Macaskill P, Steyerberg EW, et al. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): Explanation and elaboration. Ann Intern Med. 2015 Jan 6;162(1):W1–73.

CHAPTER 7

7. Annexes

Annex 1. Ethical clearance from Instituto Universitário Egas Moniz

Comissão de Ética EGAS MONIZ

Proc. Interno nº 887

Ex.mo Senhor
João Albernaz Santos Coelho das Neves

Monte de Caparica, 30 de abril de 2020

Ex.mo Senhor,

Em resposta ao Pedido de Parecer que submeteu à apreciação da Comissão de Ética da Egas Moniz, com o tema denominado **"Predição do sexo e da idade através de medidas dentárias e radiográficas: elaboração e validação de modelos forenses para a população Portuguesa"**, foi aprovado por unanimidade.

Com os melhores cumprimentos,

A Presidente da Comissão de Ética da Egas Moniz

Profª. Doutora Maria Fernanda de Mesquita

Annex 2. Data gathering clearance from Instituto Universitário Egas Moniz

INSTITUTO UNIVERSITÁRIO EGAS MONIZ

Recolha de Dados

Código | IMP.EM.EI.07_05

Curso / Laboratório Associado / Clínica Dentária Egas Moniz / Outro	Doutoramento em Ciências Biomédicas	**Ano Letivo**	2019/2020
Unidade Curricular / Serviço/Outro	Não Aplicável	**Ano / Semestre**	2º/1º
Estudante(s) / Docente /Profissional/Investigador	João Albernaz Santos Coelho das Neves		

Docente Responsável / Investigador Responsável	Prof. Doutor José João Mendes			
Contactos	**email**	jmendes@egasmoniz.edu.pt	**Telefone**	212956800

Local de recolha de dados	Clínica Dentária Egas Moniz
Morada	Morada Campus Universitário, Quinta da Granja, 2829-511 Monte de Caparica, Almada
Período de recolha	Abril 2020 - Abril 2021
Área Científica do projeto	724 Ciências Dentárias
Título do projeto	Predição do sexo e da idade através de medidas dentárias e radiográficas: elaboração e validação de modelos forenses para a população Portuguesa
Objetivo(s) da investigação	Objectivo: desenvolvimento e validação de um modelo de predição do sexo e idade considerando medidas dentárias
Resumo do projeto	A necessidade de estimativa de idade aumentou nos últimos anos devido a um aumento no número de cadáveres não identificados e a existência de indivíduos vivos que não têm uma prova válida da data de nascimento. A avaliação do envelhecimento dentário pode ser dividida em duas categorias: alterações do desenvolvimento que ocorrem na dentição humana aquando da erupção dentária e alterações degenerativas que ocorrem quando os dentes se começam a desgastar. A erupção do terceiro molar ocorre entre os 17 e os 19 anos. Este dente pode estar completamente desenvolvido mas impactado ou pode estar completamente ausente, sendo que apenas com recurso a radiografias se pode documentar de forma exata o seu estadio. A determinação do sexo é também uma subdivisão muito importante da Medicina Dentária Forense, que desempenha um papel fundamental na identificação dos indivíduos desconhecidos. Esta pode ocorrer por quatro métodos: análise do crânio; análise de DNA usando tecidos orais; morfologia dentária e através da medição das dimensões mesiodistais e vestíbulo linguais, sendo este o método mais simples para a determinação do sexo.
Metodologia de recolha de dados	A estimativa da idade dentária será realizada de acordo com o método de Cameriere et al., com recurso à avaliação de ortopantomografias. As extremidades apicais das raízes do terceiro molar inferior esquerdo de cada

	indivíduo serão analisadas e o índice de maturidade do terceiro molar, I3M, calculado, sendo definido da seguinte forma: se o desenvolvimento radicular do terceiro molar estiver completo, isto é, as extremidades apicais das raízes estiverem completamente fechadas, I3M = 0, caso contrário, I3M é calculado a partir da razão entre a soma das larguras das margens internas dos dois ápices e o comprimento do dente. Se o valor de cutoff de I3M for superior a 0,08, o indivíduo é considerado menor de 18 anos, se igual ou inferior a 0,08 então é considerado maior de idade. A criação e validação dos modelos preditivos será efectuado através de análises dos modelos de gesso; serão realizadas medições pelo mesmo operador (JN) com um paquímetro digital, de aproximação a 0,01 mm. As distâncias mesiodistal e vestibulolingual de cada dente serão medidas na maior distância. A posição do paquímetro digital deverá ser perpendicular à superfície oclusal do dente medido. Após criação do modelo que melhor distingue o sexo na população estudada, será posteriormente validada numa população subsequente e diferente da utilizada para o desenvolvimento do modelo preditivo. A Base de Dados será codificada pelo Gestor de Base de Dados do IUEM, estando a equipa de investigação cega relativamente à identificação das amostras. O tratamento e análise dos dados serão efetuados através do programa SPSS (versão 24). Utilizando-se estatística descritiva e inferencial. O nível de significância será estabelecido nos 5%.

Assinatura do docente responsável / Investigador responsável interno ou externo		Data	21/04/20

Eu, Docente Responsável / Investigador Responsável, confirmo que as Declarações de Autorização necessárias para o estudo foram entregues:

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Declaração da autorização da entidade externa - Anexar declaração em papel timbrado ou com o carimbo da instituição, devidamente datado e assinado o qual deve sustentar o preenchimento da entidade externa	- - - - -	☐
Autorização para realização de trabalho de projeto final do Mestrado Integrado de Medicina Dentária na Clínica Dentária Egas Moniz	IMP.CDEM.05_Zz[i]	☐
Autorização para realização de trabalho no âmbito do Mestrado Integrado de Ciências Farmacêuticas na Clínica Dentária Egas Moniz	IMP.CDEM.06_Zz[i]	☐

INSTITUTO UNIVERSITÁRIO EGAS MONIZ

Recolha de Dados

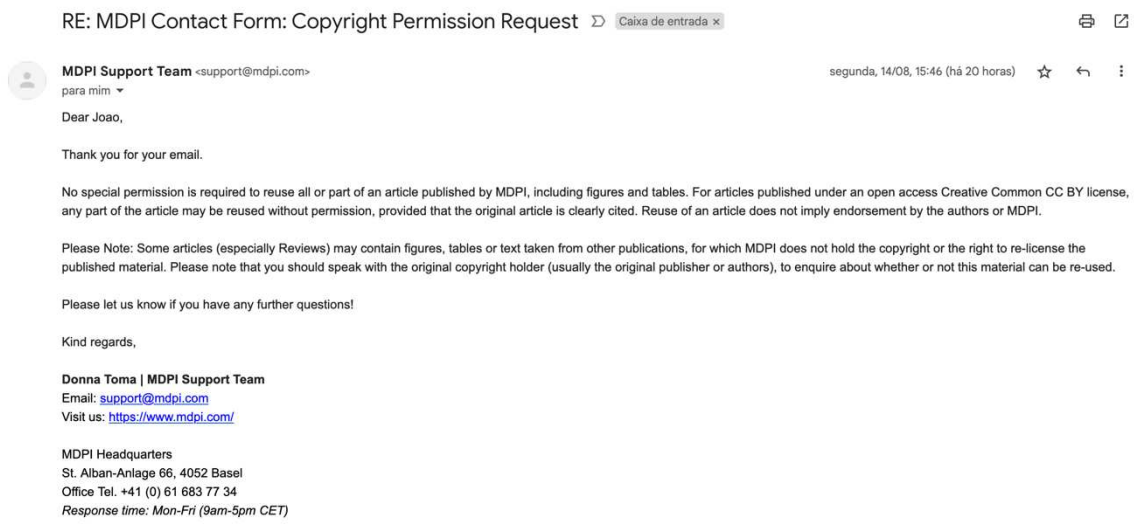
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Declaração da Direção Clínica para cedência de dentes do BDH para realização do trabalho de projeto final do Mestrado Integrado de Medicina Dentária - devidamente assinada pela Direção Clínica.	IMP.CDEM.04_Zz[i]	☐

Data 21/04/2020

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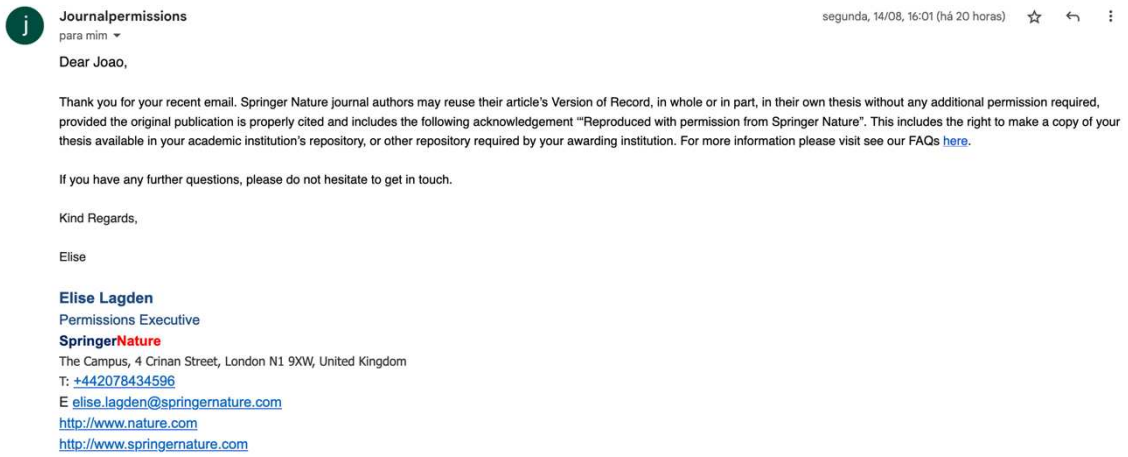
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João Albernaz Neves

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nas I Jornadas Científicas Universitárias e Politécnicas Egas Moniz, nos dias 23 e 24 de abril de 2021

PROF. DOUTOR JOSÉ GRILLO
Presidente do Conselho
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COM O ALTO PATROCÍNIO DE SUA EXCELÊNCIA — O Presidente da República

CERTIFICADO

João Albernaz Neves participou como autor do Poster,
Vanessa Machado, João Botelho, Nathalie Antunes-Ferreira, Luís Proença, Alexandre Quintas, José João Mendes, Ana Delgado participou como coautor do Poster,

com o título "**Validação do Índice de Maturação do Terceiro Molar a Nível Mundial: Revisão da Literatura**" na 3.ª *rRACS, 2020*, organizada pela Rede Académica das Ciências da Saúde da Lusofonia, nos dias 25, 28 e 29 de setembro de 2020, por videoconferência, em Portugal.

Coimbra, 29 de setembro de 2020

Comissão Organizadora da *3.ª rRACS, 2020*

Prof. Doutor António Almeida Dias
(Presidente)

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