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Environmental Constraints on Skeletal Development:
A case-study from 18th Century, Lisbon (Portugal)

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Abstract

Age-at-death (AAD) estimation is a cornerstone in Biological Anthropology, used together with other parameters to establish a demographic and social profile of archaeological samples. Methods used to estimate AAD are based on the notion that chronological age corresponds to biological age, and that the latter can be directly inferred from maturational stages.

Stress induced changes (SIC) can be used as indicators of an individual's general state of health (such as periosteal reactions (PR) and linear enamel hypoplasias (LEH)) and as markers for several diseases and physiological stress. Physiological stress may also delay bone diaphysis growth and/or epiphyseal fusion, used as age indicators.

The present dissertation had three main aims:

- to assess the reliability of different AAD estimation methods through the comparison of the estimates given by dental development (DD) and bone development (BD) methods, allowing to explore intra-individual variation.
- to explore the possibility that external factors, such as the individual's general state of health, diet, and socioeconomic status (SES), could be influencing the results obtained.
- to assess if there was any correspondence between SIC rates and the possible age indicators (IND) discrepancies observed.

The sample used in this dissertation comprises 11 immature skeletons from the Hospital Real de Todos os Santos (HRTS) archaeological collection (18th Century, Lisbon, Portugal). A total of fifty BD methods and six DD methods were used. Data obtained from these methods was compiled into individual based diagrams to allow within and between methods comparisons and additional data incorporation specific to each individual (e.g., pathological assessment via the observation of SIC).

The results revealed that BD methods tended to underestimate age when compared to the DD methods. On the other hand, most BD methods that gave similar AAD estimates to DD methods used samples from different geographical origins, from the 21st Century and with low to middle socioeconomic status. The individuals from the HRTS sample also showed a high rate of SIC (especially LEH and PR).

AAD assessment should combine a multiple methodological approach integrating BD and DD methods, pathological assessment, and an evaluation of chronic levels of stress in order to minimize age assessment errors.

Keywords: Age at death; bone development; dental development; stress induced changes; age indicators

Resumo

A estimativa da idade à morte é um parâmetro de grande relevância na Antropologia Biológica e Bioarqueologia. Este parâmetro é usado para determinar a idade dos indivíduos de coleções arqueológicas onde a idade à morte não é conhecida, auxiliando na caracterização paleodemográfica da população estudada, contribuindo assim para um entendimento mais profundo das suas dinâmicas sociais, econômicas e biológicas.

Os métodos utilizados para realizar a estimativa da idade à morte baseiam-se na ideia de que a idade cronológica de um indivíduo corresponde diretamente à sua idade biológica. Essa correspondência é inferida através dos estágios de maturação que ficam gravados nos seus remanescentes biológicos no momento da morte. Estes estágios de maturação incluem diferentes fases de desenvolvimento observadas tanto no esqueleto humano quanto na dentição. No esqueleto, considera-se o crescimento linear dos ossos, o aparecimento de centros de ossificação primários e secundários, e a fusão epifisária. Na dentição, são considerados o desenvolvimento e a erupção dos dentes. Estes últimos são amplamente utilizados, uma vez que fornecem um vislumbre de forma relativamente precisa sobre a idade biológica de um indivíduo.

No entanto, fatores externos aos indivíduos podem influenciar os diversos indicadores etários utilizados. Esses fatores podem fazer com que a progressão dos estágios de maturação não seja sempre linear, como tem sido tradicionalmente representada na literatura. É essencial considerar a variação intra-individual, uma vez que diferentes indivíduos podem experimentar diferentes ritmos de crescimento e desenvolvimento, influenciados por fatores externos, tais como: nutrição, estado geral da saúde individual e coletiva, stresse fisiológico e condições socioeconômicas.

Alterações induzidas por stresse fisiológico podem servir como indicadores do estado geral de saúde de um indivíduo. O stresse fisiológico pode retardar o crescimento linear das diáfises de ossos longos e/ou os tempos de fusão epifisária, que são tradicionalmente usados como indicadores etários. É importante notar que o stresse fisiológico não afeta apenas o crescimento linear do esqueleto, mas também causa alterações nos ossos e dentes, tais como hipoplasias lineares do esmalte, *hiperostose porótica*, *cribra orbitalia*, *cribra humeralis*, *cribra femoralis* e determinadas reações do periósteo, que podem ser observadas em osso seco, mesmo após a decomposição do periósteo.

A etiologia destas alterações não é extensivamente documentada devido à dificuldade em estabelecer relações causais, sendo muitas dessas ligações ainda desconhecidas. A conexão entre *cribra orbitalia* e *hiperostose porótica* com anemia é a mais explorada, sugerindo que esta última será a causa mais provável destas alterações. No entanto, cada caso individual deve ser avaliado como único, e o estudo da distribuição de alterações induzidas por stress fisiológico no esqueleto é necessário para a realização de um diagnóstico diferencial.

Ao estimar a idade à morte, é importante considerar até que ponto a variabilidade populacional e individual pode afetar estas estimativas, já que não existem métodos universais de estimativa da idade com altos níveis de precisão e fiabilidade. Como mencionado anteriormente, o stresse nutricional e/ou fisiológico pode ter um grande impacto na saúde e desenvolvimento do indivíduo. Consequentemente, isso pode afetar a idade cronológica estimada com base no estágio de desenvolvimento do indivíduo, seja subestimando (em casos de desenvolvimento retardado) ou superestimando (em casos onde alterações ósseas associadas a processos degenerativos são usadas para estimar a idade). Esse viés impacta os resultados obtidos tanto na Antropologia Biológica quanto na Forense, distorcendo o perfil demográfico, impedindo a identificação positiva de um conjunto de restos humanos ou fornecendo uma estimativa de idade incorreta em indivíduos vivos.

Assim sendo, uma ligação direta entre os estágios de desenvolvimento de um indivíduo e a idade cronológica pode nem sempre ser observada. A avaliação do estágio de maturidade de um indivíduo no

momento da morte não deve ser interpretada como um vínculo direto com a idade cronológica, pois pode não fornecer uma estimativa precisa em todos os casos. Em vez disso, deve ser interpretada como uma coleção de informações sobre a história de vida do indivíduo. Estudos sobre o desenvolvimento humano devem ser realizados não apenas com o objetivo de estimar a idade à morte, mas também para entender o ambiente em que o indivíduo se desenvolveu até o momento da sua morte. O paradoxo osteológico também deve ser considerado, já que indivíduos que sucumbem a uma morte prematura (excluindo casos de assassinato, acidentes e doenças súbitas) podem não ser representativos dos padrões de desenvolvimento da população total. Nesse sentido, a estimativa da idade à morte pode beneficiar de uma abordagem mais holística, em que os indicadores de idade são observados juntamente com marcadores de stresse fisiológico. Isso proporcionará uma leitura mais abrangente do desenvolvimento do indivíduo ao longo da vida, permitindo uma estimativa mais fiável da idade à morte e favorecendo uma abordagem biocultural ao estudo dos restos humanos.

A relevância do presente estudo está ligada à crescente necessidade de estimar a idade em indivíduos (vivos e falecidos), acompanhada pelo aumento do número de métodos de estimativa de idade disponíveis. Esses métodos precisam de ser testados para entender sua fiabilidade e precisão quando aplicados a indivíduos cuja história de vida é desconhecida. A presente dissertação testou isso através da comparação entre as estimativas de idade à morte fornecidas por métodos baseados no desenvolvimento ósseo e dentário. Já que o desenvolvimento dentário tende a ser menos afetado por fatores externos e apresenta menores taxas de variação entre populações, as estimativas de idade obtidas a partir desse desenvolvimento podem ser comparadas às fornecidas pelo desenvolvimento ósseo. A hipótese era de que essas estimativas seriam consideravelmente diferentes, indicando que a taxa de desenvolvimento ósseo pode diferir da taxa de desenvolvimento dentário. Isso também implica que as taxas de desenvolvimento ósseo da amostra presente são diferentes das amostras usadas para desenvolver os métodos de estimativa de idade à morte.

Essa análise foi complementada pela comparação entre as características de cada método (origem geográfica das amostras, cronologia, status socioeconômico e metodologias empregadas), para verificar se há alguma tendência entre os métodos de desenvolvimento ósseo que forneceram estimativas de idade à morte semelhantes aos métodos de desenvolvimento dentário, e a existência de alterações induzidas por stresse. A hipótese era de que indivíduos com mais discrepâncias entre as estimativas de desenvolvimento ósseo e dentário também apresentariam um número maior de alterações induzidas por stresse. Nesse caso, a existência dessas alterações poderia ser usada como um indicador de stresse fisiológico durante a vida, o que pode traduzir-se num grande impacto no desenvolvimento ósseo.

A amostra utilizada na presente dissertação era formada por 11 conjuntos de remanescentes ósseos humanos da coleção arqueológica do Hospital Real de Todos os Santos (Lisboa, séc. XVIII). Ao todo, foram utilizados cinquenta métodos de estimativa da idade à morte baseados no desenvolvimento ósseo e seis baseados no desenvolvimento dentário. Os valores obtidos foram transformados num diagrama para cada indivíduo para permitir a comparação entre os diferentes métodos aplicados e com os dados relativos à presença de alterações induzidas por stress.

Os resultados revelaram que os métodos baseados no desenvolvimento ósseo tendencialmente subestimavam a idade à morte dos indivíduos quando comparados com os métodos baseados no desenvolvimento dentário. A maioria dos métodos que forneceram estimativas semelhantes aos métodos de desenvolvimento dentário baseava-se em amostras com diferentes origens geográficas, datadas do século XXI e com estatuto socioeconómico considerado entre baixo e médio. Isso sugere que o contexto socioeconômico e o estado geral de saúde dos indivíduos podem ter um impacto significativo na precisão dos métodos de estimativa da idade à morte.

Os indivíduos da presente amostra apresentavam elevadas taxas de alterações induzidas por stresse, nomeadamente hipoplasias lineares do esmalte e reações do periósteo. Essas alterações indicam

que os indivíduos sofreram níveis significativos de stresse fisiológico durante a vida, o que pode ter influenciado o seu desenvolvimento ósseo e dentário.

Com base nesses resultados, conclui-se que a estimativa da idade à morte deve ser baseada numa combinação de métodos de desenvolvimento ósseo e dentário, análise patológica e uma avaliação dos níveis crónicos de stresse fisiológico. Esta abordagem combinada pode minimizar o erro associado à estimativa da idade à morte e fornecer uma imagem mais precisa e completa das condições de vida e saúde das populações antigas.

Em suma, a estimativa da idade à morte é uma ferramenta crucial na Antropologia Biológica e na Bioarqueologia. No entanto, é necessário considerar a influência de fatores externos e individuais para aumentar a precisão das estimativas. A combinação de diferentes métodos e a análise de indicadores de stresse fisiológico poderam, assim, contribuir significativamente para compreensão mais profunda e detalhada das populações antigas.

Palavras-chave: Idade à morte; desenvolvimento ósseo; desenvolvimento dentário; alterações induzidas por stress; indicadores etários.

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Abbreviations List

AAD	Age at Death
IND	Age Indicators
BA	Biological Age
BD	Bone Development
CA	Chronological Age
CF	*Cribra femoralis*
CH	*Cribra humeralis*
CO	*Cribra orbitalia*
DD	Dental Development
HRTS	Hospital Real de Todos os Santos
LEH	Linear Enamel Hypoplasia
PH	*Porotic Hyperostosis*
PR	Periosteal Reaction
SES	Socioeconomic Status
SIC	Stress Induced Changes
GnRH	Gonadotropin-releasing hormone
FSH	Follicle-stimulating Hormone
LH	Luteinizing Hormone
GH	Growth Hormone
IGF-1	Insulin-like Growth Factor 1
GH/IGF-1	Growth Hormone/Insulin-like Growth Factor 1 complex
WHO	World Health Organization

1. Introduction

Age-at-death (AAD) estimation is a cornerstone of Biological and Forensic Anthropology. It is a key point when establishing an individual's biological profile, together with biological sex estimation, stature and populational affinities, as well as the pathological profile. Estimating AAD has also shown itself useful in other disciplines, such as: human biology, paediatrics, human evolution, paleopathology, human palaeontology, legal medicine, and public health (Alemán et al., 2012; Liversidge, 2015). AAD can be useful for characterising past populations and its paleodemography, in archaeological samples. It is also used in forensic contexts to establish a positive identification of human remains and to estimate the age of living individuals (in cases of murder trials, migration, refugee seekers, child pornography and establishment of majority for legal responsibility). Forensic sciences have, thus, been a major lever for the expansion of AAD methods seen in the last decade, mainly due to the increase in migration movements and asylum seekers without any/valid documentation (Ekizoglu et al., 2015, 2019a, 2019b; Hillewig et al., 2013; Hocaoglu et al., 2020; Krämer et al., 2014; Milenkovic et al., 2013; Oldrini et al., 2016; Schaefer et al., 2015; Ubelaker, 2010; Ubelaker & Khosrowshahi, 2019).

AAD can be estimated based on two different parameters: bone development (BD) and maturation, and/or dental development (DD) and eruption. However, when estimating age it is necessary to consider the effects of factors external to individual development, such as diet, socioeconomic status (SES) and general state of health, and how these may impact BD and DD, as well as how this may impact the accuracy of the methods employed (Cardoso, 2007; Cunha et al., 2009; Frisancho et al., 1970b, 1970a; Gluckman et al., 2005; Schmeling et al., 2000, 2006b). Several authors to recommend the use of sex- and population-specific methods, to accommodate for these differences (Al-Qtaitat et al., 2016; Boccone et al., 2010; Cardoso, 2008a, 2008b; Coqueugniot & Weaver, 2007; Kocasarac et al., 2016; Kotěrová et al., 2018; Marera & Satyapal, 2018; Milenkovic et al., 2013; Oldrini et al., 2016; Sullivan et al., 2017). Nevertheless, individual variation also plays an important role in development and should be considered. DD has been shown to be less influenced by environmental factors, presenting a strong genetic correlation (Cardoso, 2007; Frisancho et al., 1970a, 1970b; Schmeling et al., 2000, 2006b).

This dissertation's primary aim was to test the reliability of different AAD estimation methods based on BD, when compared to DD methods. To accomplish this, fifty BD methods and six DD methods were tested on a set of human remains from an archaeological collection. Its secondary aim was to explore the influence of other factors on BD methods' performance for AAD estimation, such as individual variability and/or environmental influence (such as population's general state of health, diet, and SES). If an AAD assessment is not reliable nor accurate, this may have consequences: in Biological Anthropology, it may impact the characterisation of a demographic profile; in Forensic Anthropology, it could hinder individual identification or lead to legal implications by obstructing accurate legal decisions. This dissertation intended to call for awareness to the importance of an individual-based assessment, considering not only traditional age indicators (IND), but also the general state of health of the individual. The reliability of AAD estimates may increase if the differences between maturational stages, biological age (BA) and chronological age (CA) are recognized, while also providing the tools for a better understanding of how individual and/or environmental factors affect human development.

1.1. A Brief History of AAD estimation methods

AAD estimation of human remains has a relatively long history and has evolved together with technological and medicine advancements. During the 19th Century, the main focus was on the macroscopic assessment of different anatomical structures (such as cranial sutures closure, dental development and eruption, appearance of secondary ossification centres, bone size and epiphyseal union) to estimate AAD (Liversidge et al., 2015; Ubelaker, 2010; Ferembach et al., 1980; Masset, 1989; Meindl & Lovejoy, 1985). During this period, the variability in timings of cranial sutures closure and third molar development and eruption was brought to attention by several authors, who cautioned for their use as IND and stating that CA "can rarely be given with any great accuracy" (Dwight, 1878) when based on these structures. Despite these critics, the use of cranial sutures and the third molar to infer CA has continued to this day (Alhadi et al., 2019; Dorandeu et al., 2008; Fan et al., 2020; Hens & Godde, 2020; Li et al., 2022; Nikolova et al., 2019, 2021; Upreti, 2019), and have consequently been criticised for their limitations and associated error (Ruengdit et al., 2020; Sivakumaran, 2014).

Throughout time, the focus on these structures shifted to different IND, influenced by different scientific advancements and statements. These include:

- The understanding that applying several AAD estimation methods to the same individual increased the estimate's accuracy.
- The recognition that different anatomical regions provide different information and AAD estimates (e.g.: in immature individuals, DD and BD may provide important information regarding age, but these elements are not useful to estimate AAD of a biologically mature individual - see subchapter 1.4. for an explanation on maturation).
- The recognition of individual variation during growth and its impact on age estimation and associated errors (Cunha et al., 2009; Schmeling et al., 2000, 2006b, 2007; Ubelaker & Khosrowshahi, 2019).

These three statements revealed a gap in AAD methods, exposing the need for additional research based on larger, and more diverse samples, able to include factors such as individual variability, sexual dimorphism, and age-related bone changes. This would reduce samples' bias, as well as decrease AAD estimation methods' associated errors and allow the development of more complex and sophisticated methods (Cunha et al., 2009; Frisancho et al., 1970b, 1970a; Schmeling et al., 2000, 2006b, 2007; Ubelaker, 2010; Ubelaker & Khosrowshahi, 2019).

Human skeletal remnants' preservation and conservation states are also of key importance to determine which AAD estimation methods should be applied. This has led to a growth of studies on the impacts of taphonomy on human skeletal remnants during the last decade since human remains are rarely found in pristine conditions in archaeological contexts. Fragile anatomical regions (such as cranial sutures and the pubic symphysis) are the first ones to succumb to taphonomic changes and the damage inevitably caused by archaeological interventions. This led investigators to search for new INDs (Ubelaker & Khosrowshahi, 2019), hoping to minimise the limitations taphonomic issues could have on AAD assessment. Instead, they turned to anatomical regions that were more reliable and more resistant to post-mortem damage. This included an assessment of skeletal development and maturation in immature individuals based on tooth development and eruption, long bones measurements, secondary ossification centres development and epiphyseal union (AlQahtani et al., 2010; Cardoso, 2008a, 2008b; Coqueugniot & Weaver, 2007; Corron et al., 2019; Olivares et al., 2014; Olivares & Aguilera, 2017; Pérez et al., 2017; Rissech et al., 2013; Ubelaker, 1989), and an evaluation of degenerative processes in mature individuals, such as morphological changes observed in the pubic symphysis and auricular surface (Alves-Cardoso & Assis, 2018; Buckberry & Chamberlain, 2002; Calce, 2012; Lovejoy et al., 1985; Mays, 2012). Bone histology also proved to be a very reliable and accurate tool for AAD estimation. However, this technique is still not frequently used, since it is time consuming and needs

specialised training for sample preparation and structure interpretation (Streeter, 2010; Ubelaker & Khosrowshahi, 2019).

As mentioned above, the influence of individual and/or environmental factors on development also started to be the focus of several studies. Although every country in the world presents its own spectre of poverty and SES, these aspects are very influenced by global geopolitics and economy. Hence, most low- to middle-income populations are associated with African, Asian, and South American countries. This may result in a lower SES of the population and even on a poor nutritional status, due to the lack of access to proper diet and health resources. On the other hand, middle- to high-income countries are usually associated with European and North American countries and are linked to a higher SES and an improved nutritional status. Based on this, and the fact that population variation has an important genetic component, various authors have suggested the use of population-specific AAD estimation methods (Boccone et al., 2010; Olivares & Aguilera, 2017; Pérez et al., 2017).

Methods based on dentition's metric traits have also been developed. These methods reported high accuracy values and a decrease in observer's subjectivity - measures offer a more objective assessment of dentition than visual observation of dentition's development and eruption. Some of these methods are based on parameters such as root and/or crown measurements and an evaluation of pulp/tooth ratio, and its application does not require specific training or equipment (Cameriere et al., 2013; Cardoso et al., 2019; D'Ortenzio et al., 2018; Fernandes et al., 2011; Halilah et al., 2018; Viciano et al., 2018).

Technological and mathematical advancements were also important for the development of more objective AAD methods. 3D scanning and mathematical approaches such as multivariate adaptive regression splines, Bayesian statistics and transition analysis decreased the subjectivity associated with observational techniques for AAD estimation (Bullock et al., 2013; Corron et al., 2019; Godde & Hens, 2012; Kotěrová et al., 2018; Stoyanova et al., 2017; Ubelaker & Khosrowshahi, 2019; Villa et al., 2013). Biomechanical analyses are also more objective and accurate, but present several setbacks: these methods are more time consuming and need specific equipment and specialised training for sample preparation and structure interpretation (Adserias-Garriga et al., 2018; Freire-Aradas et al., 2016; Ubelaker & Khosrowshahi, 2019).

Despite all these advancements in AAD methods in the last decades, the absence of a standardised research design and variable coding is still an important setback, since it influences the methods' accuracy and prevents comparisons between different studies. Besides introducing a bias on the results, this lack of standardisation may also cause different studies based on the same sample to produce different results. For example, authors that use different age categories may obtain different results, even when using the same sample (Lopreno et al., 2013).

1.2. From immaturity to maturity

Age-at-death (AAD) estimation methods can be divided into two major categories, depending on bone and dental maturation stages. Individuals' classification based on AAD estimation can either be described, in general terms, as immature and/or mature individuals. The concept of immaturity defines individuals in whom maturation has not reached a stopping point, comprising every stage of development from foetal development to the final stage of dental development and eruption, and epiphyseal fusion. Hence, immature individuals refer to those in which maturation has not finished in some (or all) of the anatomical regions of the skeleton. In these cases, age indicators (IND) used to estimate AAD comprise tooth mineralisation and eruption (AlQahtani et al., 2010; Arthanari et al., 2020; Irurita et al., 2014; Ubelaker, 1989), length of long bones (Boccone et al., 2010; Cardoso et al., 2016; Primeau et al., 2016; Rissech et al., 2013), development of primary and secondary centres of ossification

and epiphyseal fusion (Cardoso, 2008a, 2008b; Cardoso & Ríos, 2011; Coqueugniot & Weaver, 2007; Hillewig et al., 2013; Krämer et al., 2014; Schaefer et al., 2015; Wittschieber et al., 2013). By opposition, mature individuals are those in which maturation has reached a stopping point, and age can no longer be predicted based on developmental stages (Liversidge, 2015). In these cases, AAD estimation is based on degeneracy (defined by the decrease in cartilage integrity of different skeletal regions – mainly articulations; for example, the reabsorption of the cartilage between the vertebral bodies is associated with the appearance of changes of pathological nature such as osteophytes and schmorl's nodes, with different studies showing a correlation with age in mature individuals (Wang et al., 2012)). These also include the evaluation of the degree of dental wear (Lovejoy, 1985; Prince et al., 2008), observations of bone changes of the head and sternal ends of ribs (DiGangi et al., 2009; Işcan et al., 1984), observation of morphological changes (such as micro and macroporosity) of the auricular surface and pubic symphysis (Brooks & Suchey, 1990; Buckberry & Chamberlain, 2002; Calce, 2012; Lovejoy et al., 1985; Mays, 2012), degree of cranial sutures' obliteration (Fan et al., 2020; Masset, 1989; Ruengdit et al., 2020) and identification of skeletal changes of pathological nature usually associated with advancing age (e.g.: osteoarthritis, osteophytes, ossification of the thyroid and cricoid, and entheseal changes) (Brennaman et al., 2017; Chiba et al., 2022; Garvin, 2008; Kacar et al., 2017; Listi & Manhein, 2012; Praneatpolgrang et al., 2019; Watanabe & Terazawa, 2006). Regardless of the methods, a cautionary approach is always necessary since many changes express more than the cumulative effect of age. For example, osteoarthritis, osteophytes and entheseal changes may be secundary to other pathologies (e.g., trauma), or related to biomechanical constraints linked to activities undertaken during life, which may promote the acceleration of these degenerative processes (Alves-Cardoso & Assis, 2018; Lopreno et al., 2013).

For any IND (either the ones used in immature or in mature individuals) to be considered reliable for AAD estimation, they must respect several principles. These include: they need to be universal and complete (present in every individual of similar chronological age and in both biological sexes); they must occur in the same order and in regular intervals; they must distinguish between different levels of maturity in individuals of the same chronological age; they should present good intra- and inter-observer reliability; and lastly, they must reflect a real change related to maturation instead of an individual variation or a simple variation in size (Cameron, 2015).

AAD estimation methods must also meet several criteria in order to be considered valid. They must have been presented to the scientific community through a publication in peer-reviewed scientific journals, they must present a high accuracy and indicate it in a clear and direct manner and, when estimating age in the living, the methods should consider additional medical ethics and legal concerns (Ritz-Timme et al., 2000).

Several manuals dedicated to the study of human osteological remains have aggregated many of the main AAD methods used, serving as a guide to training bioanthropologists (Buikstra & Ubelaker, 1994; Katzenberg & Saunders, 2007; White & Folkens, 2005).

1.3. The importance of Documented and Anatomical Human Osteological Collections for AAD estimation

The increase in the number of AAD estimation methods seen in the last decades was possible mainly due to the development of identified/documented skeletal remains and anatomical collections (Alves-Cardoso & Campanacho, 2022; Henderson & Alves-Cardoso, 2018). This is due to the type of information available for each set of human remains from these collections, that in most cases include: name, date and place of birth, date and place of death, AAD, cause of death, biological sex, occupation

at time of death, etc. Forming an AAD method based on these collections allows for the control of these variables, reducing the inherent error in age estimation and increasing accuracy, while testing several hypotheses of bone development and maturation. However, this information may still be biassed. Problems such as self-reported age, social differences in the way chronological age (CA) is perceived, SES, age of first job, clinical history, periods of poverty and famine throughout life, hobbies, among others, are not described in the information provided for each individual, but may still have a considerable influence in their development. Moreover, these collections should always be considered a sample and not a true and faithful representation of the society they derive from (Alves-Cardoso & Henderson, 2013; Henderson & Alves-Cardoso, 2018).

These collections exist in almost every continent (for an overview of the global distribution of these collections see Alves-Cardoso and Campanacho (2022)). However, most of these collections are housed in North America and Europe, as well as some countries of South America and South Africa. This may be influenced by the countries' developmental rate, which is linked to government investments in scientific research; countries with a higher development index tend to have a higher investment in technologies and scientific research than the ones with lower development indexes. The Iberian Peninsula houses some of the largest collections, with Portuguese collections being the most cited in literature, especially the osteological collection from Coimbra University (Alves-Cardoso & Campanacho, 2022). Due to the remains' high rate of completion and preservation, these types of collections have been used for a considerable number of studies, including those focusing on AAD estimation (Campanacho et al., 2018; Cardoso, 2008a, 2008b; Cardoso et al., 2013, 2014; Cardoso & Ríos, 2011; Cardoso & Severino, 2010; Coqueugniot & Weaver, 2007; Olivares & Aguilera, 2017; Pérez et al., 2017).

When a sample from an identified osteological collection is subjected to a study aiming to form an AAD estimation method, a correlation between skeletal changes and CA is sought. In this sense, CA is inferred through BA which is, in its turn, inferred through maturational stages. However, as previously mentioned, these collections may have a bias regarding individual's information, which may represent a setback in the accuracy of the results obtained (Henderson & Alves-Cardoso, 2018). Another important factor to consider is that skeletal maturity does not always have a direct correspondence to CA (i.e. different individuals with similar CAs may present different maturation stages, and vice-versa), which could influence the method's accuracy and reliability (Liversidge et al., 2015; Márquez-Grant, 2015). This is also why AAD estimates are not provided as a specific age, but rather as an age range (Hillewig et al., 2013).

1.4. Estimating age in the living

The need to estimate age in the living has been a decisive factor for the increase in age estimation methods during the last decade. This is due to the current socio-political context, with an exponential growth in migration movements and in the number of asylum-seekers (in cases where there is no identification documents, or these are not considered valid), but also to other legal issues, such as child pornography and human trafficking (in which a positive identification of the individual is intended), and the assessment of age for legal responsibility (Chowdhuri et al., 2018; Ekizoglu et al., 2015, 2019a; El-Din et al., 2019; Galić et al., 2016; Gurses & Altinsoy, 2021; Hillewig et al., 2013; Krämer et al., 2014; Mahon et al., 2018; Milenkovic et al., 2013; Oldrini et al., 2016; Schaefer et al., 2015; Torimitsu et al., 2019; Wittschieber et al., 2013; Zhang et al., 2015). For each case, the age threshold for legal responsibility and/or adulthood depends on the country's laws. The most common age thresholds of legal relevance are the attainment of 18 and/or 21 years of age, and to determine these individuals' age there is a need for accurate and reliable methods (Schmeling et al., 2007; Ubelaker & Khosrowshahi,

2019). This led to a rapid increase in the number of methods available and to the rectification of previously existing ones (Franklin, 2010).

In cases in which age needs to be estimated in the living, imagery techniques are applied (aiming to observe the development of primary and secondary centres of ossification), together with the assessment of visual physical parameters: height, weight, number of teeth, the degree of gait, musculature, skin elasticity and tone, amongst others. The most common imagery technique used is X-ray, to assess the degree of dental, hand, and wrist development (Cunha et al., 2009; Gelbrich et al., 2015; Schmeling et al., 2007). This evaluation aims to pinpoint the developmental stage (skeletal, sexual, and dental maturation) of the individual, as well as the occurrence of maturational events (such as menarche, spermarche, voice change, peak height velocity and attainment of 95% of mature high), which are considered essential to determine if an individual has reached maturity. When collecting these data, cultural and personal subjectivity regarding timings of development and varying concepts such as adulthood need to be considered. In legal cases, it is also important to acknowledge that the maturational stage does not translate into an exact chronological age (CA), which prevents an exact estimate from being provided. In these cases, it is recommended that estimates be done in the form of an age range, while also providing the most probable age (Cameron, 2015; Liversidge, 2015; Márquez-Grant, 2015).

Using an X-ray of the hand and wrist to evaluate bone maturation enables the simultaneous observation of several ossification centres and stages of development of the distal ulna and radius' epiphysis. Even though an observation of different anatomical structures usually allows for a more reliable age estimate, individual variation is an important factor that may influence the accuracy of that estimate. This variation appears to be more visible between the ages of 14 and 16 years, in the case of hand and wrist X-rays (Lira, 2018; Urschler et al., 2015). More recently, different imagery techniques such as CT scans and MRI, as well as the use of different anatomical regions, such as the medial clavicle and the iliac crest have been used to develop new age estimation methods (Chowdhuri et al., 2018; Ekizoglu et al., 2015; Hillewig et al., 2013; Lottering et al., 2017; Mahon et al., 2018; Marera & Satyapal, 2018; Milenkovic et al., 2013; Torimitsu et al., 2019; Wittschieber et al., 2013; Zhang et al., 2015).

Age estimation methods based on X-ray of dentition allows for an evaluation of the stage of dental development and eruption based on a population-specific method. Although dentition has been shown to be less affected by external factors, there is still a considerable degree of cultural and individual variation, specifically when evaluating the maturation of the third molars. In some cases, a completely mature third molar root can be observed by the age of 16 years, while in others, this may only happen by the age of 23 years. This presents a difficulty when it comes to distinguishing between individuals of an older CA and individuals with a later maturation stage. Despite the uncertainty introduced by such variation, tooth development evaluation is still a useful and one of the most reliable tools for age estimation (AlQahtani et al., 2010; Cardoso, 2007; Carneiro et al., 2017; Olivares et al., 2014; Liversidge, 2015).

Although there has been a long ongoing discussion regarding how to proceed when estimating age in living individuals, a consensus about the use of medical and non-medical features has not yet been reached. Sypek and colleagues (2016) tested a set of less invasive methods on asylum-seekers in Australia, including the report of life stories (as told by the children themselves and their families), assessment of physical and bone maturation and dental development. The combination of all these parameters resulted in highly accurate CA estimations. Considering this outcome, the authors recommend a holistic approach when it comes to estimating age on the living to ensure reliable assessments, while also acknowledging the influence of external factors, such as genetics and environment.

Currently, methods developed on human skeletal remains are being used in living individuals and vice-versa. However, individuals who died during development are more likely to have succumbed to a

wide degree of diseases or due to periods of sub- and/or undernutrition, not leading the normal course of life (excluding cases of murder, accidents, and sudden diseases). This may also mean that, in these cases, development did not occur in optimal conditions, increasing the chances of disruptions. In living individuals, this may also be the case when considering asylum-seekers, since these tend to flee from countries where health and food conditions are subpart, but it may not be the case for the remaining individuals. This may increase the error of age estimate, since methods based on individuals developing in strenuous conditions may be currently applied to individuals developing under optimal conditions and vice-versa, without consideration on how this may influence their development rates (Frisancho et al., 1970b, 1970a; Gluckman et al., 2005; Schmeling et al., 2000, 2006a, 2006b; Ubelaker & Khosrowshahi, 2019).

1.5. Environmental constraints on skeleton/bone and teeth development

As previously described, growth and sexual maturation are very commonly used as age indicators (IND). However, these processes cannot be considered linear since they are influenced by several external factors. These factors range from genetic variability to bone changes of pathological nature, cultural and socio-economic variability, exposure to pollutants and contaminants, access to education, balanced nutrition, physical activity, body mass index, amongst others. All these factors may introduce variation to the maturation processes, whether it is in terms of timings, order and/or occurrence (Beaumont et al., 2018; Márquez-Grant, 2015; Merritt, 2015, 2017).

Growth includes two major periods that alternate between each other: periods of saltation, in which linear growth is visible and measurable; and periods of stasis, in which there is no measurable growth of the individual. Both are present during the development of the human body. However, the normal progression of these stages may be impaired by factors such as nutritional stress, caused by malnutrition (inadequate intake of certain vitamins, minerals and/or other nutrients) and/or sub nutrition (insufficient food consumption). In these situations, growth may cease leaving the individual in a prolonged period of stasis. This affects skeletal growth to a higher extent than it does dental development. In the long bones, during a period of stasis there is a cessation of calcium deposition, which means that there is a reduction or complete absence of linear growth. On the other hand, dentine deposition continues during these periods, although some studies have shown a slight delay in dental development in cases of malnutrition and/or subnutrition (Cardoso, 2007; Liversidge, 2015). Development may return to normality if adequate nutrition is restored, allowing for a "catch-up growth" (Beaumont et al., 2018; Beaumont & Montgomery, 2015; Frisancho et al., 1970a, 1970b).

If proper nutrition is not restored, the state of malnutrition and/or sub nutrition can become chronic. When this happens during growth, the immune system's development may be compromised, leaving the individual in a permanent state of frailty throughout life. This may result in chronic inflammation and infection, as well as in a permanent small stature (stunted growth), which is even more prominent in females (Beaumont et al., 2018). Individuals who present stunting in later stages of development tend to be born with a lower weight and size than expected, showing slow linear growth rates during childhood and puberty. As previously explained, stunting is characterised by prolonged exposure to low quality foods (sub nutrition) and/or in insufficient quantities (undernutrition), not providing enough nutrients, vitamins, minerals and proteins for the normal development of the body (Campisi et al., 2018). In a state of chronic nutritional stress, the human body tends to conserve energy, giving priority to metabolic processes essential for its survival and neglecting linear growth (which directly affects long bone length and, consequently, individual's height) and sexual maturation. Several studies have also suggested that epiphyseal fusion timings are less affected by nutritional stress than linear development of long bones (which are majorly responsible for the individual's height). If an individual is in a

continuous stage of stasis for a prolonged period, catch-up growth can only happen until the complete fusion of the epiphyseal plates. After this, the potential for linear growth disappears. In this way, height can, theoretically, be considered as a measure of health and quality of nutrition in a given population, even in adult individuals (Cardoso, 2007; Frisancho et al., 1970a, 1970b; Schmeling et al., 2000, 2006b).

Malnutrition and subnutrition have been shown to be important external factors on sexual and skeletal maturation. There is also a direct effect on the endocrine system which, in turn, will affect its interaction with the growth hormone (essential for the occurrence of peak height growth and sexual maturation during adolescence). Malnutrition or sub nutrition can cause delays in the signalling for Gonadotropin-releasing hormone (GnRH) production by the hypothalamus, which, consequently, will cause the anterior pituitary gland to produce lower levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The two latter hormones are directly responsible for the production of sexual hormones in males (testosterone) and females (oestrogen). Therefore, if LH and FSH present lower levels than what is expected, the outset of sexual cycles will not be promoted, resulting in later sexual developments (Campisi et al., 2018; Christian & Smith, 2018). Malnutrition and sub nutrition are found to be two of the main reasons for the appearance of the menarche at a later medium chronological age (CA) in girls. It is also one of the reasons for the decrease in menarches' age in modern societies, due to the improvement of healthcare and easier access to a more balanced nutrition. In addition, the growth hormone/insulin-like growth factor 1 (GH/IGF-1) complex is stimulated by the increase in hormone production during puberty and is very sensitive to hormonal changes. Hence, if this hormone production process does not occur inside the normal parameters, the GH/IGF-1 complex won't be stimulated. This will noticeably influence linear growth of bone diaphysis. Low levels of IGF-1 are also associated with a deficiency of vitamin A and B6, zinc, magnesium, and potassium (Hintz et al., 1978; Martins et al., 2017).

Puberty and maturation are biological and sociocultural periods of great importance, comprising visible physical changes. However, as above mentioned, these processes do not occur independently from external factors, such as the environment in which a person is developing. Such factors influence not only timings, but also the normal occurrence of these processes. Menarche is an example of a maturational event affected by external factors. In the case of girls inserted in more disadvantaged social environments, menarche tends to occur at a later chronological age than in girls from higher socioeconomic classes. This may be due to a connection between the notion of low SES and a more limited access to education, proper nutrition and healthcare, explaining this delay. This can also be the case in high performance athletes and models, due to physical strenuous conditions imposed by intense physical activity and nutritional restrictions, which can be a cause of physiological stress. As such, skeletal changes associated with puberty tend to follow general growth trends. Bone growth accelerates until it reaches peak height velocity, which usually occurs during adolescence. Afterwards, it decreases until the process of tooth eruption, linear growth and epiphyseal fusion is complete. During this process, secondary sexual characteristics are also developing (namely pubic hair, the increase in size of testicles and penis in males and of breasts in females). With all these events being connected with each other, when one is disturbed by external factors, the normal progression of the others will also be compromised (Henderson & Padez, 2017).

In some cases, the differences between a child developing under optimal conditions and another developing in more trying ones can be abysmal. However, in both cases females tend to reach maturation earlier than males. This may be suggestive of some degree of sexual dimorphism during development, manifesting the need for sex-specific age estimation methods. This also stresses the importance of acknowledging the age ranges presented in most AAD estimation methods, which is usually of ± 2 years. Since biological sex can not be estimated until the fusion of the *os coxae*, AAD estimation before this event occurs independently from sexual diagnose. This means that the periods of time attributed to each

stage of BD in boys and girls should be combined, which will result in larger age intervals, and will, in turn, increase its accuracy.

Six considerations can be withdrawn from these observations: there are significant differences between maturation stages and chronological age; maturation is established based on maturity indicators (recognizable phases that occur during a continuous process); there is variation within each individual; during each of the maturational processes, different structures may not be in the same developmental stage (e.g.: during sexual maturation, genitalia and pubic hair may be in different stages of development); there is sexual dimorphism during development, with girls usually reaching later stages of maturation at an earlier CA; stature does not necessarily relate to maturation and growth process (for a visual example and more information on these considerations, see Cameron (2015)).

During puberty, there is the accumulation of the largest amount of calcium in bones (around 40% of the total amount), which comprises about one third of the bones' mineral section. Thus, bone mineralization is directly dependent on the intake of calcium and other minerals, such as phosphorus and magnesium. Bone mineralization is also influenced by other factors, such as the degree of physical activity and genetics. Based on this knowledge, there is the possibility that the onset for puberty is caused by bone maturation, since children normally enter puberty when they have reached a certain maturation stage (there is a known relation between the attainment of puberty and peak height velocity, which usually occur in similar stages). Bone maturation ends when the epiphyseal plates finish their fusion, reaching an end point on the individual's linear growth (Cardoso, 2007; Frisancho et al., 1970a, 1970b; Schmeling et al., 2000). Despite a possible state of chronic malnutrition, every individual eventually enters the puberty phase, reaching a certain reproductive capacity and linear growth, even if this happens at a considerably later age. Thus, in a situation of chronic malnutrition, it is possible that growth is suppressed to conserve nutrients for other essential needs, maintaining the body's homeostasis when facing adverse conditions. This reinforces the idea that linear growth is postponed, but is not irreversibly lost (Campisi et al., 2018; Christian & Smith, 2018).

A study performed by Ammann and colleagues (2000) on rats fed with a low-calorie diet with different levels of casein showed a gradual decrease in bone mineral density over the weeks, affecting the trabecular bone at first and the entire skeleton after some time in every experimental group (this was even more notorious in the group of rats fed with the lowest level of casein). The decrease in bone mineral density is associated with significant changes in biomechanical properties, affecting anatomical regions such as the lumbar vertebrae, neck of the femur and the diaphysis of the tibia. This leads to a decrease in resistance to adversities and biomechanical stress, in skeletal rigidity, as well as in the amount of energy absorbed. In humans, similar problems are recurrent in elderly people in whom a nutritional deficiency has been documented (a clear example of this are hip fractures, due to a lower resistance to biomechanical stressors, such as falls). However, this low protein intake can also be associated with other pathologies such as anorexia nervosa, that leads to a disturbance of sexual hormones and consequent decreased bone mineral density, or even osteoporosis. Hence being, Ammann and colleagues (2000) concluded that a situation of malnutrition (low-calorie and protein-deficient diet) decreases bone mineral density in both trabecular and cortical bone and is potentially related to a decrease in IGF-1 and/or oestrogen levels (Ammann et al., 2000).

A longitudinal study by Matkovic and colleagues (2004) performed on human subjects aimed to understand the consequences of long-term calcium and dairy consumption on bone mineral density of the os coxae, vertebrae, ulna and radius during development. The study followed these individuals from age twelve until adulthood, with regular visits in which an X-ray of the previously mentioned anatomical regions was taken. Results showed a positive influence of calcium supplementation and consumption of dairy products in bone mineral density of the os coxae, ulna, and radius. Consumption of dairy products was also associated with higher bone mineral density in the vertebrae. This may be due to calcium exerting its action on bone growth mainly through volumetric bone mineral density, while dairy products

may have an additional impact on periosteal bone growth and expansion due to its more complex nutritional composition (including other minerals and vitamins, as well as protein and lipidic composition). This resulted in a significant difference in volumetric bone mineral density when comparing the groups who had a high dairy products intake and who were administered with calcium supplementation, which may suggest a more favourable impact of dairy products on bone growth during childhood. These results may also be influenced by external factors to the experiment, such as physical activity. Calcium and protein intake may be crucial to the growth peak recorded during childhood and adolescence, affecting bone growth and maturation. It can be concluded that adolescence is a critical period in which an inadequate intake of calcium and protein can be detrimental to skeletal maturation.

Malnutrition impairs not only individual growth and maturation, but also economic growth by perpetuating poverty. Improper nutrition during growth is associated with the development of chronic noncommunicable diseases, which may have a direct impact on the population's working capacity. In 2020, the World Health Organization (WHO) estimated that around 149.2 million children under 5 years of age showed evidence of stunting and around 50 million were underweight for their CA. 79% of these children are from countries of low- and low-to-middle income and suffered from malnutrition and/or sub nutrition. This may bear consequences for the health of these individuals, presenting more vulnerable immune systems and a lower life expectancy (World Health Organization, 2021). All these factors pose obstacles when estimating age in immature individuals, as they may affect the accuracy of the results obtained. Reduced linear growth in adolescence leads to reduced height in adulthood and its causes are still poorly understood, although the relationship with malnutrition is apparently obvious. These factors don't only influence skeleton development, but also the general state of health and frailty, leaving definitive marks throughout life (Cardoso, 2007; Christian & Smith, 2018; Frisancho et al., 1970a, 1970b; Martins et al., 2017).

Nowadays, children between the ages of 10 and 19 years constitute the largest generation of young people ever, with around 1.8 billion around the world. However, 90% live in countries of medium or low average income. Diseases amongst these populations derive mainly from infectious or accidental causes, but nutritional deficiencies and reduced linear growth are public health problems of growing significance. Girls are usually in a more vulnerable position in their social contexts, representing a considerable part of the population at risk. Health and nutritional well-being of these individuals depends directly on diet patterns, physical activity, education, and social norms regarding young marriages. Nutritional requirements, including protein, iron, calcium, and other nutrient intake, are essential to fulfil the energy demand that happens in adolescence to support the increased rate of growth, peak height velocity, and proper development. Endocrine factors are essential to promote the normal growth of adolescents, making them vulnerable to malnutrition (Christian & Smith, 2018). This generation represents the future of human resources, economic growth, and reproductive potential. In developed countries, the average age at which menarche occurs in girls decreased markedly and is currently between 10 and 11 years, due to optimised hygiene conditions, access to quality education and food. However, in developing countries, malnutrition is prevalent, with living conditions playing a more substantial role in adolescents' growth and sexual maturation (Campisi et al., 2018).

It is also important to notice that physiological stress does not only affect the linear growth of the skeleton. It is also credited to cause bone and teeth stress-induced changes (SIC), such as: linear enamel hypoplasias (LEH), *cribra orbitalia* (CO), *cribra humeralis* (CH), *cribra femoralis* (CF), *porotic hyperostosis* (PH) and surface bone changes commonly referred to as periosteal reactions (PR) - the latter results from the changes observed in the thin layer of conjunctive tissue that covers the bone, called periosteum: after death, this layer is decomposed, but some marks of these changes can still be observed in dry bone (White & Folkens, 2005). Bibliography establishing the aetiology of the previously mentioned changes is not extensive due to the difficulty this entails, with most of these causal links remaining unknown. The connection between CO and PH with anaemia is the one that has been most

explored, with enough evidence suggesting that anaemia is a strong probable cause of these SIC, but every individual case needs to be assessed as unique, and the study of SIC distribution throughout the skeleton needs to be performed so that a differential diagnosis can be drawn. Nevertheless, a strong connection between SIC and environmental influences during development is suggested (Beaumont et al., 2018; Beaumont & Montgomery, 2015; Brickley, 2018; Chaichun et al., 2021; Mangas-Carrasco & López-Costas, 2021; Nakayam, 2019; O'Donnell et al., 2020; Pilloud & Schwitalla, 2020; Reed et al., 2017; Rinaldo et al., 2019; Towle & Irish, 2020; van Schaik et al., 2018).

1.6. Comparing skeletal and dental age

In cases where chronic sub- and/or undernutrition causes a delay in skeletal development, there is a significant difference between maturation stages on the skeleton and dentition, which will result in two different estimates for CA. This happens because, as previously explained, timings of tooth formation and eruption appear to be less influenced by external factors. Dentition is, thus, more reliable when inferring CA in immature individuals. In this way, measuring the difference of age estimates between BD and DD may also constitute a method to evaluate the rate of influence of external factors on the individual's development (Beaumont et al., 2018; Buckberry, 2015; Cardoso, 2007; Carneiro et al., 2013; Liversidge, 2015).

Several studies still suggest that a smaller delay in DD exists in cases of poverty and malnutrition. This delay is not relevant to the point of nullifying these methods but should be taken into consideration. Although this variation in the timings of dental development may not be significant, physiological stress can be observed in dentition through other visible changes, such as linear enamel hypoplasia (LEH). Hypoplasias appear in the tooth enamel during crown development due to a diminished dentine deposition, usually associated with a period of nutritional stress. These observations suggest that small variations in tooth development may hide marks of physiological stress, aggravated due to external factors - notwithstanding cases of congenital defects. The tooth showing the biggest degree of population variation is the third molar, with root closure and eruption occurring in a varying age range (eruption can occur between 12 and 23 years old, and in some cases does not occur). Hence, age estimation based on DD and eruption is more accurate in individuals showing earlier DD maturation stages (preferably prior to third molar's eruption and apex closure). Since every individual has its own maturational scale, there is an inherent difficulty in inferring CA from the maturational stage. Therefore, there is a necessity for a more rigorous approach, combining BD and DD methodology for a more accurate estimate of age (Beaumont et al., 2018; Cardoso, 2007; Carneiro et al., 2017; Liversidge, 2015; Nakayam, 2019; Reed et al., 2017; Towle & Irish, 2020).

In sum, population variation has a higher genetic influence, while individual variation is more affected by environmental factors. External factors, alongside with individual and population variation, play important roles in skeletal maturation and may affect the accuracy of chronological AAD estimation. On the other hand, methods for estimating age in adults present their own setbacks and limitations. In the case of adults, since maturation has reached a stopping point, AAD is inferred through indicators associated with skeleton degeneracy. However, degeneracy processes, much like developmental processes, are multifactorial, being influenced by factors other than age. Mays (2015) described how different anatomical regions, such as the pubic symphysis, auricular surface (sacroiliac joint) and the lumbar vertebrae, may suffer from external factors that may not be directly related to age. Sexual hormones and biomechanical efforts can develop degenerative processes in young individuals. Deficiency in vitamin D, energy balance, reproductive, biomechanical, and genetic elements may also influence these indicators. For example, degenerative changes linked with the hyaline cartilage of the pubic symphysis are usually associated with older individuals. However, these changes may also occur

during pregnancy, due to the release of hormones such as relaxin, which increase joint laxity. Multiparous females may also present these types of changes due to the release of oestrogens, which induce bone resorption at the pubis, and due to the mechanical forces of parturition. The same alterations at the pubic symphysis were observed in athletes and cases of trauma. Another example is vitamin D deficiency, which may lead to inadequately mineralized bone since normal levels of vitamin D are essential to establish the pathways for calcium deposition. When estimating AAD, it is important to consider the extent to which population and individual variability may affect those estimates since there are no universal AAD estimation methods with high levels of accuracy and reliability.

1.7. Consequences for age estimation

As mentioned in the previous section, nutritional and/or physiological stress may have a great impact on the individual's health and development. Consequently, this may impact the CA estimated based on the individual's developmental stage, either by underestimating (in cases of delayed development) or overestimating it (in cases where bone changes associated with degenerative processes are used to estimate age). This bias will impact the results obtained both in Biological and Forensic Anthropology, by skewing a demographic profile, by preventing a positive identification of a set of human skeletal remains or by providing a wrong age estimate in living individuals.

A direct link between an individual's developmental stages and CA may not always be observed. That is, the assessment of where an individual was in their path to maturity at the moment of death should not be interpreted as a direct link to CA, since it may not provide an accurate AAD estimate in every case. It should rather be interpreted as a collection of information on the individual's life history. Studies on human development should not only be performed with the aim of estimating AAD, but also as a means of understanding the environment in which the individual was developing at time of death. The osteological paradox (Wood et al., 1992) also needs to be considered, since individuals who succumb to a premature death (excluding cases of murder, accidents, and sudden diseases) may not be representative of the total population developmental patterns. In this sense, AAD estimation may benefit from a more holistic approach, in which IND are observed together with physiological stress markers. This will provide a more comprehensive reading of the individual's development through life, allowing for a more reliable AAD estimation: one that favours a biocultural approach to the study of the human remains.

The present study's relevance is linked to the growing necessity for estimating age in both living and dead individuals, accompanied by an increasing number of available AAD methods. The methods available need to be tested, to understand their reliability and accuracy when applied to individuals in whom life history is unknown. The present dissertation tested this through the comparison between AAD estimates provided by methods based on BD and DD. Since the latter is tendentially less affected by external factors and presents lower rates of variation between populations, AAD estimates obtained from this can be compared to the ones provided by BD. It was hypothesised that these estimates would be considerably different, meaning that there may be a different BD rate than DD. This also means that the present sample's BD rates are different from the samples used to develop the AAD estimation methods.

This was complemented with the comparison between each method's characteristics (samples geographical origin, chronology, socioeconomic status (SES), and methodologies employed), to see if there is any trend between the BD methods that provided similar AAD estimates to the DD methods, and with the existence of SIC. It was also hypothesised that individuals with more discrepancies between BD and DD estimates would also present a higher number of SIC. In this case, the existence of SIC could be used as an indicator of physiological stress during life, that may have a great impact in BD.

2. Methodological Approach

As abovementioned, the aim of the present dissertation was to assess the reliability of different age at death (AAD) estimation methods based on bone development (BD) on a sample of human skeletal remains of unknown AAD. To achieve this, BD was compared to dental development (DD), which would allow for the study of intra- and inter-individual variation within a small sample of skeletal human remains. The assessment of bone changes of pathological nature, with a special focus on stress-induced changes (SIC) aimed to see if there was a coexistence between a higher number of SIC and age indicators (IND) discrepancies in each individual. Although this analysis is not enough to provide a cause-effect relation, it allowed to explore the hypothesis that external factors, such as the individual's general state of health, diet and SES, could influence AAD estimates.

Previous studies had already presented a preliminary analysis of the biological profile of this sample and its relation to historical context (Laughton, 2015; Alves-Cardoso et al., 2013; Casimiro, 2020). The methodological approach included different steps, aiming for the (re)assessment of the human skeletal remnants' biological and palaeopathological profile.

Firstly, the human remains were re-assessed at Laboratório de Antropologia Biológica e Osteologia Humana (LABOH). Each individual's information useful to establish skeleton completeness and preservation state, as well as the biological profile, was systematically recorded, to build a database for AAD estimation. An extensive photographic record of the human skeletal remains was carried out between the months of July and September 2020, to facilitate working from home due to Covid-19 restrictions.

The totality of the HRTS archaeological sample included 17 individuals (Casimiro, 2020), including individuals in which maturation was complete (i.e., in which dental and skeletal development had reached an end point) and individuals in which maturation was not complete (i.e., individuals in which dental and/or skeletal development was still occurring at time of death). Considering the aims of the present dissertation, only the information regarding the second group of individuals was used for the following steps. These individuals were identified by the stratigraphic units (SU) assigned during excavation and included [573], [575], [872], [1008], [1214], [1296], [1313], [1314], [1406], [1419] and [1429], compiling a total of 11 sets of human skeletal remains.

2.1. Biological and Paleopathological Profile

Following data compilation at LABOH, each individual's biological and pathological profile was estimated, which included:

1. **Biological sex estimation**. Biological sex was only assessed in individuals in whom the three bones of the os coxae were fused, to reduce the interpretative error associated with biological sex assessment on individuals in whom sex dimorphism is still incipient (Cardoso & Saunders, 2008; Galdames et al., 2009; Rogers, 2009). In the cases where the three bones of the os coxae were not fused, individuals were considered as "Indeterminate". To estimate the biological sex of this set of human skeletal remains, different features of the os coxae and skull (Bruzek, 2002; Buikstra & Ubelaker, 1994) and osteometric parameters (Wasterlain, 2000), - such as the long bones maximum length, vertical diameter of the femur's and humerus' head, among others - were used, and were given preference by the order in which they were described above.
2. **Bone maturation stage.** Each individual's bone maturation was characterized through parameters such as: stage of epiphyseal union (if it was at 0%, 25%, 50%, 75% or 100%,

and if there was an epiphyseal scar visible), long bone measurements, dental development and eruption and morphology of skeletal regions considered useful for AAD estimation (such as the pubic symphysis and auricular surface) (White & Folkens, 2005; Buikstra & Ubelaker, 1994; Ortner, 2003).

3. **Bone changes of pathological nature.** Lastly, the sample was analyzed for the presence of skeletal changes of pathological nature, especially stress induced changes (SIC): linear enamel hypoplasia (LEH), *porotic hyperostosis* (PH), *cribra orbitalia* (CO), *cribra humeralis* (CH), *cribra femoralis* (CF) and periosteal reactions (PR) (presence of woven and/or lamellar bone) (Figure 2.1.1.).

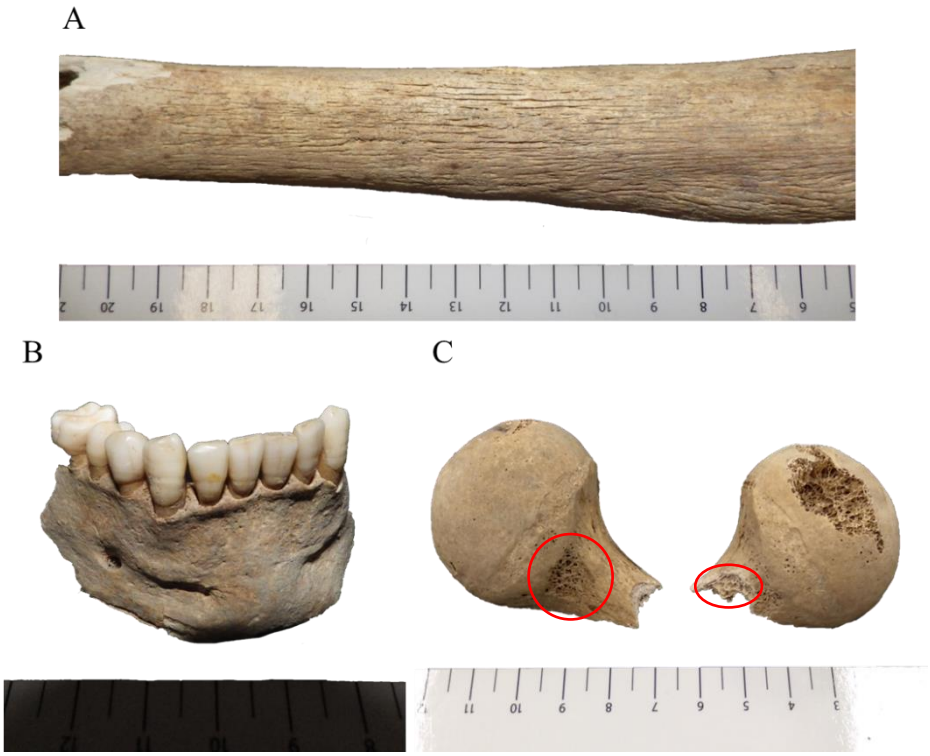

Figure 2.1.1.: *Exemplificative photos of SIC found in the HRTS sample: A – PR in the medial view of the left distal tibia's diaphysis, individual [1313]; B - LEH on the mandibular dentition in individual [1314]; C - CF present on right and left femurs' necks of individual [573].*

2.2. AAD estimation methods

A total of fifty-six methods were selected from various searches in two different databases - Google scholar and Scopus -, based on keywords such as "age-at-death", "age-at-death assessment" and "age-at-death assessment immature individuals". The AAD estimation methods included fifty methods based on BD (Appendix A: Table A.1.) and six methods based on DD (Appendix A: Table A.2.). BD methods were published between 2007 and 2020, while DD methods were published between 1963 and 2020.

The goal of this dissertation was to evaluate how accurate BD methods could be when estimating AAD. Since the sample used was of archaeological origin, chronological AAD was unknown, so BD was compared to DD. However, not every individual from the sample was recovered with the corresponding dentition. DD methods were only applicable to individuals with preserved dentition, which included: [575], [1008], [1214], [1296], [1314], [1406], and [1429]. Hence, the sample was divided into two groups: the group of individuals with dentition preserved (Group_Dentition) and the

group of individuals without dentition preserved (Group_No_Dentition) - see Figure 2.2.1. for each group composition. In Group_Dentition, BD was compared to DD. The BD methods that gave a similar AAD estimate to DD in a higher number of individuals from this group was used as the control group of methods in Group_No_Dentition (replacing the DD methods as the reference methods). The information from the BD methods from these two groups was pooled, to compare their parameters.

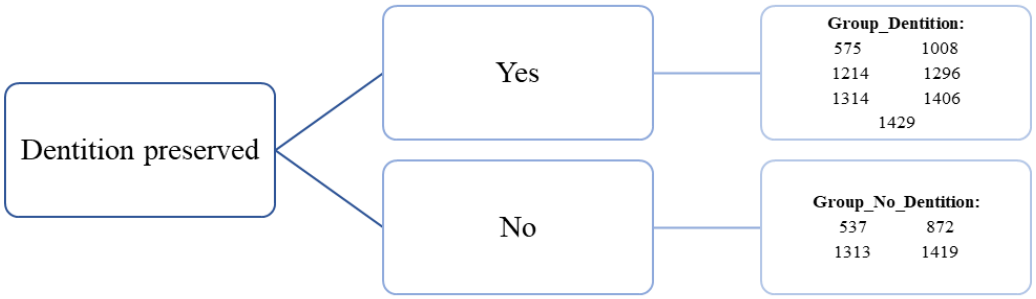

Figure 2.2.1.: *Composition of Group_Dentition and Group_No_Dentition.*

Then, BD methods were applied to each individual. A diagram was created for each individual to represent the age estimates obtained by each method (for an example, see Figure 2.2.2). This was used as a visual aid to determine which BD methods gave similar AAD estimates to the DD methods. In Figure 2.2.2., the number attributed to each AAD estimation method is represented in the vertical axis while the horizontal axis represents the age range provided by each method. The bars in a darker shade of blue represent the age range provided by the DD methods applied, and the black rectangle represents the age range provided by the intersection of every DD method. The black rectangle provided the age range for each one of the seven individuals from Group_Dentition, to which the estimates obtained based on the BD methods (represented in light blue) were compared.

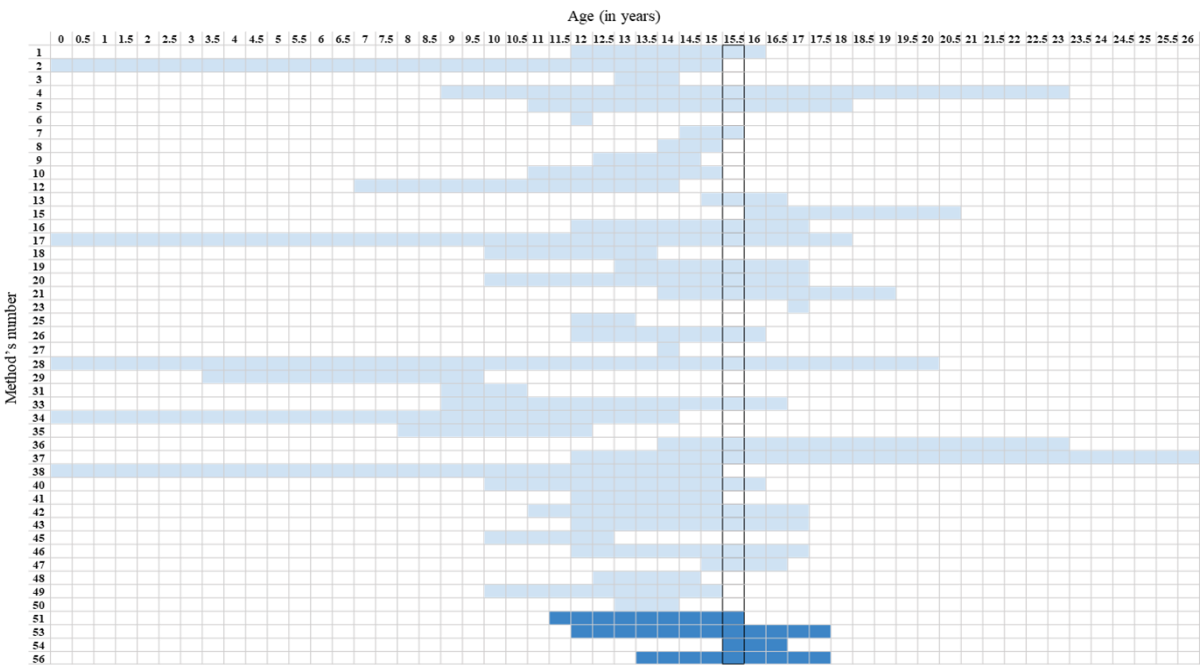

Figure 2.2.2.: *Diagram representing the AAD estimate provided by every method applied to individual [575]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which every DD method overlap.*

Next, BD methods that gave a similar AAD estimate to the DD methods were pooled, and their parameters were compared. In individuals in which dentition was not preserved ([573], [872], [1313] and [1419]), DD methods could not be used as reference for comparison with the BD methods. In these cases, the BD methods that gave a similar AAD estimation to the DD methods a higher number of times in the group of individuals with dentition preserved were considered as the reference methods for the group of individuals without dentition preserved. After this, the information from these BD methods was pooled, to see if and/or which of these methods had similar characteristics: if the methods used samples with similar geographical origins, chronology, size, age and sex distribution and SES, as well as study materials, anatomical region(s) and methodological approaches employed. Table A.1. is an example of how these informations were synthesised from the AAD estimation methods selected.

Figure 2.2.3. is similar to Figure 2.2.2., except that the bars in a darker shade of blue correspond to the reference BD methods obtained from the group with dentition preserved (as previously explained, the methods that gave a similar AAD estimate to the DD methods in the group of individuals with dentition preserved). The bars in a lighter shade of blue represent the age ranges provided by each BD method.

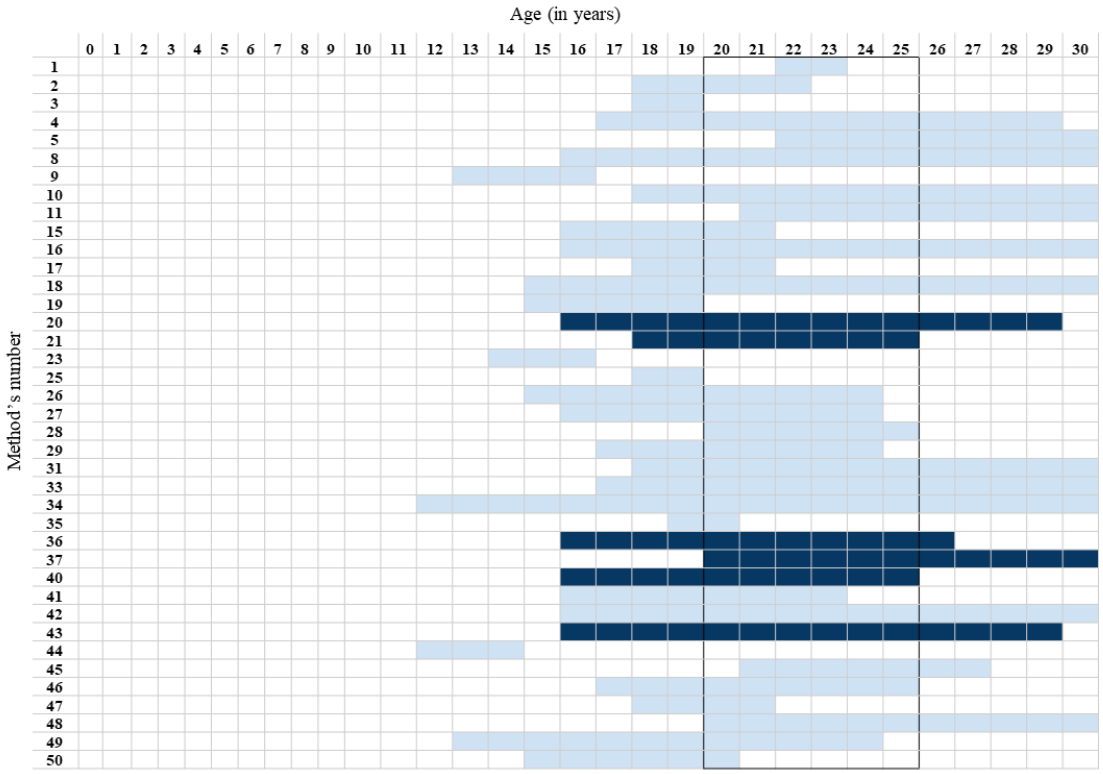

Figure 2.2.3.: *Diagram representing the AAD estimate provided by every method applied to individual [573]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis provides each method's number. The AAD estimate provided by the reference BD methods is represented by the bars in dark blue, and the bars in light blue represent the AAD estimate provided by the remaining BD methods. The black contour marks the age interval in which every reference BD method overlap.*

Following this, the total sample was again divided into two different groups (Figure 2.2.4.): the group of individuals without epiphyseal union (Group_No_Fusion) and the group of individuals in which epiphyseal union had already initiated (Group_Partial_Fusion). This division was based on the different methodology used to estimate AAD in each case: in cases where epiphyseal union has not yet begun, osteometric parameters are more frequently used; on the other hand, the stages of epiphyseal union are more useful in individuals where these events have already started. The information from these two groups was also pooled to search for similarities and/or differences between the BD methods that gave similar AAD estimates do the reference methods.

These data were compared to SIC frequency, to see if there was an overlap between the individuals that presented a higher number of SIC and AAD estimates discrepancies.

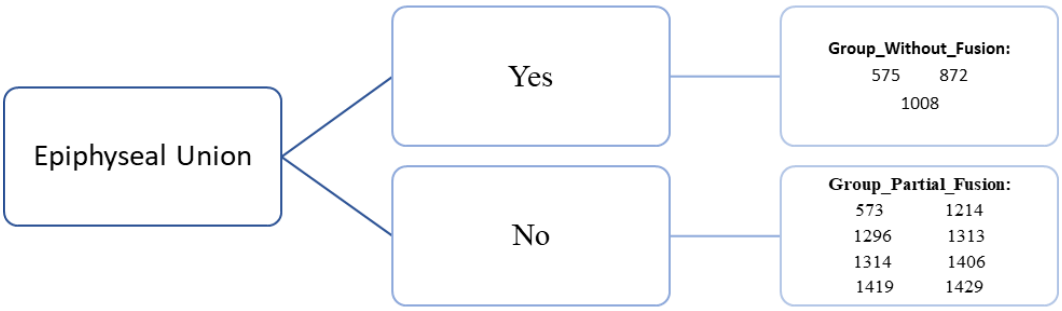

Figure 2.2.4.: *Composition of Group_Without_Fusion and Group_Partial_Fusion.*

2.3. Human remains sample

The present dissertation was based on a sample of human remains exhumed during an archaeological intervention at Praça da Figueira, Lisbon (Portugal), between the years of 1999 and 2001. The site was associated with the Hospital Real de Todos os Santos (HRTS). The sample was dated from the first half of the 18th Century. The archaeological intervention uncovered a funerary context on a former circulation corridor. This corridor is thought to have been deactivated during the structural reforms developed during King João V reign and reused for funerary purposes. The graves were oriented with the walls (NE-SW), forming two lines and a circulation space between them (Alves-Cardoso et al., 2013; Casimiro, 2021).

The sample was composed by 17 individuals, with different rates of conservation and preservation. For this archaeological sample, there was no documentation about these individuals' available. This includes geographical origin and SES, as well as AAD, biological sex and health status. Although some of these aspects can be inferred through osteological analysis (such as AAD, biological sex and health status), the remaining parameters cannot. To fill these gaps, historical and social information was considered.

The HRTS was first idealised by King João II during the 15th Century. This monarch aimed to centralise Lisbon's health care system in the capital, creating the first infrastructure run in a self-management system. The laying of the first stone occurred in 1492. Although an exact date for its opening is unknown, the first registers indicate that the hospital was already treating patients in 1501 and 1502 (Silva & Leite, 2015; Silva, 2015). The hospital presented a cruciform plant, following the trends of prominent hospitals at the time. The centre of the cross had a high altar which would be visible to the main wards, allowing hospital staff and patients to attend mass from their beds (Ramos, 1993).

HRTS was known for the treatment of several infectious diseases, such as syphilis and tuberculosis. The main demographic of its patients were the poor with curable diseases. Patients with incurable diseases were accommodated in different units (Silva, 2015). A continuous search for assistance in the hospital led to successive changes and additions. The hospital also provided hospice and shelter services to abandoned children, the poor, and pilgrims, which led to a wide variety of pathologies being treated there (Ramos, 1993).

In 1755, the earthquake that destroyed a substantial part of the city of Lisbon was also fatal to the HRTS. Efforts to reconstruct the hospital were made, but in 1769, following the remodelling plans for the city of Lisbon by Marquês de Pombal, the hospital was abandoned and dismantled. Its services were transferred to the new Hospital Real de S. José. HRTS served as a model hospital, in which the construction of several other hospitals along the Portuguese Kingdom were based, in India, Brazil and several African countries. The reuse of the land where HRTS was previously located began in 1775,

giving rise to a square, currently known as Praça da Figueira (Alves-Cardoso et al., 2013; Silva & Silva, 2017).

Over the three centuries in which the hospital was running, people with different geographical origins were admitted and treated in these quarters. The majority of the patients were of portuguese origin, but there was also a substantial percentage of hospitalised individuals from the Portuguese colonies, specially from Brazil and Africa (Laughton, 2015).

During the HRTS samples' period, the city of Lisbon dealt with various epidemic crises. This was due to poor sanitary and health conditions, as well as to a growing and shifting population caused by migratory movements. Some of the most common epidemics in Lisbon were syphilis, tuberculosis, smallpox, typhus, and dysentery (Alves-Cardoso et al., 2013; Ramos, 1993). Lisbon's population mortality also suffered from seasonal influences, peaking during the winter months, due to the cold weather. During these periods, its population was more affected by respiratory diseases. Mortality also increased during the summer, due to droughts that increased food prices and induced periods of famine (Moreira, 2008; Rijo, 2012).

Due to these conditions, child mortality affected one in every four children before they turned one year old, and only half of the children would live to complete 7 years of age. Mortality was reduced between the ages of 10 and 15 years and had a slight increase in the age group between 20 and 40 years old mainly due to tuberculosis infection. Female mortality also increased in these age range due to pregnancy and child labour. Mortality increased again in the age groups older than 50 years old. Life expectancy was low, presenting values between 25 and 35 years (Moreira, 2008; Rijo et al., 2010).

These historical data suggest that there is a high chance of finding individuals in the HRTS sample that were younger than 35 years old, either from Portugal, Brazil and African countries, presenting a low SES status. Due to prolonged periods of famine, these individuals were possibly frail and suffered from a stunted growth. It also suggests that younger individuals are more likely to be female and older individuals are more likely to be male, with a considerable chance of cause of death being one of the infectious diseases mentioned above, especially considering the hospital context associated with these human remains. The preliminary results obtained from previous studies (Laughton, 2015; Alves-Cardoso et al., 2013; Casimiro, 2020) seem to support this hypothesis: there is a higher number of individuals estimated as Female in younger categories (individuals without complete epiphyseal union) and as Male in older categories (individuals with complete epiphyseal union), but these results could be skewed due to the presence of three cases in which the biological sex was considered Indeterminate. Note that biological sex was only estimated in individuals with complete development of the os coxae and fusion of the ilium, pubis and ischium (which can happen between the ages of 14 and 19 (Schaefer et al., 2009)) to reduce the interpretative error associated with biological sex estimation in young individuals, due to the absence, or incipient, sexual dimorphism (Cardoso & Saunders, 2008; Galdames et al., 2009; Rogers, 2009). In this sense, skeletons identified as [575], [872] and [1008] were considered as Indeterminate regarding biological sex estimation. The skeletons [1313] and [1412] were also considered Indeterminate due to low preservation and conservation rates, which impeded the application of any biological sex estimation method due to the absence of the required structures. From the remaining individuals, a total of 47% (10/21) were estimated as Male and a total of 28% (6/21) as Female.

Prior studies of the HRTS sample estimated that it was mainly composed by younger individuals (in whom skeletal and/or dental maturation was incomplete) in different maturation stages (Laughton, 2015; Alves-Cardoso et al., 2013). These studies also showed that, despite the sample association with a hospital context, the human remains had a low frequency of pathological bone changes. However, these rates were still similar to other contemporary hospitals from across Europe (Laughton, 2015). This reinforces the importance of considering the osteological paradox (Wood et al., 1992), which states that, even though these individuals do not show signs of bone changes of pathological nature, this does not

mean that their death was not caused by some type of illness, and/or they were not subjected to it during life. Individuals with frail immune systems may offer less resistance to a pathogen, succumbing to death faster and without time for the bone changes to appear. To complement the bioanthropological analysis, information based on the abovementioned historical, social, and cultural context of the human remains was considered.

Considering the aim of the present dissertation - to assess the reliability of different age at death (AAD) estimation methods on a sample of skeletal human remains of unknown AAD, through the comparison of the AAD estimates given by dental development (DD) and bone development (BD) - only individuals in which maturation was not complete were considered (this included dental development and/or epiphyseal union). Hence, only 11 individuals were used in this dissertation: [573], [575], [872], [1008], [1214], [1296], [1313], [1314], [1406], [1419] and [1429]. Individuals' [575], [872], [1008] and [1313] biological sex was estimated as Indeterminate (a total of 36%). Individuals' [575], [872], [1008] lacked the fusion of the three bones from the os coxae, the individual [1313] lacked the anatomical features usable to estimate biological sex (either based on morphological or osteometric parameters). In the remaining individuals, 36% were classified as female ([573], [1296], [1406] and [1429]), and 27% (3/11) were classified as male ([1214], [1314] and [1419]).

3. Results

The present section was organised in three parts: the first one was an overall profile of the methods used; the second was a comparison between BD and DD methods in the grouped individuals (explained in section 2.1. Methods); the last part presents a detailed individual assessment, referencing what SIC were present in each set of human skeletal remains.

3.1. Overall assessment of the AAD estimation methods

As previously described in section 2.1. Methods, a total of 50 BD methods and 6 DD methods were selected. The breakdown of these results based on the criteria used to select the methods is explored below. These included: countries of sample provenance; sample chronology; sample size and composition; sample socioeconomic status (SES); study material; anatomical areas; methodological approach.

BD_Sample: 46% of the samples used were from Europe, with Portugal being the most common country of origin. This was followed by Asia (with a significant number of samples originating from Turkey), Africa and North America (USA). Only two methods used samples from Oceania (Australia) and one used a combination of samples from different continents (method 39): Europe, North America, Africa and Asia (Figure 3.1.1.).

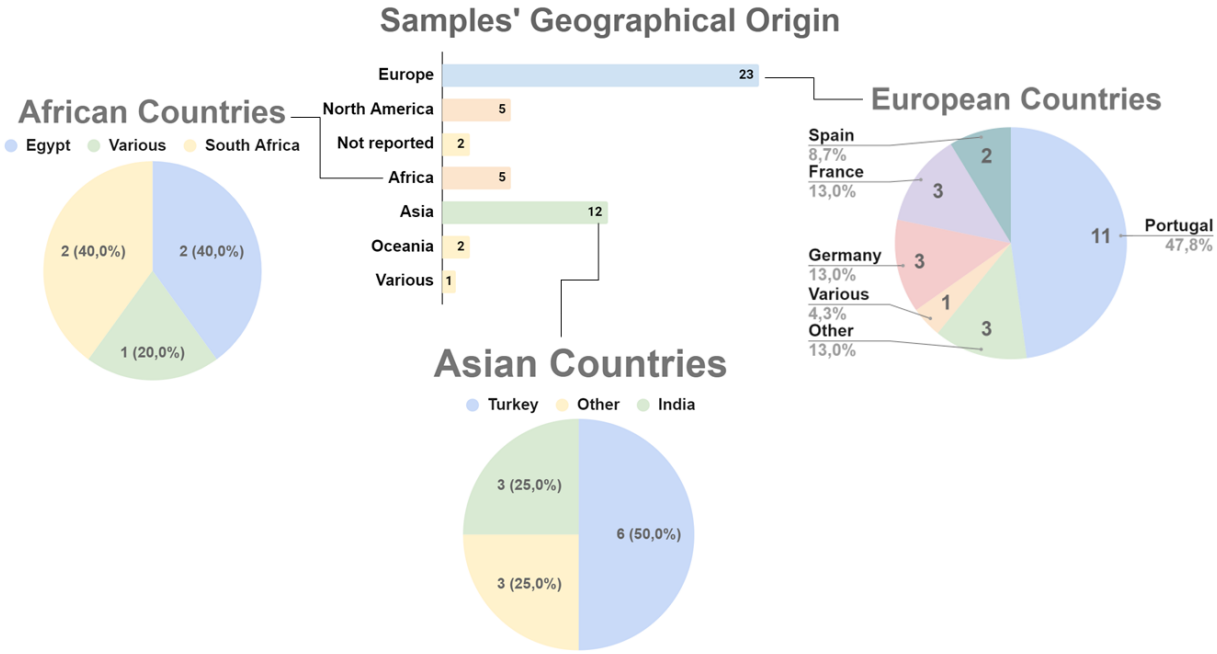

Figure 3.1.1.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods. In the centre bar graph, the continent distribution is represented, followed by the pie charts of the countries from the higher frequency continents (Europe, Asia, and Africa).*

BD_Sample_chronology: The chronology of the samples used in the method's development ranged between 2300-1750 before the current era (BCE) and the 21st Century. A total of 46% of the samples were dated from the 21st Century. Only 12% of the BD methods did not report the samples' chronology (Figure 3.1.2.A).

BD_Sample_size_distribution: Sample size from these BD methods varied between a total of 32 individuals (method 9) to 1777 individuals (method 21). Sex distribution also varied greatly in the different BD methods: females percentage ranged between 0% (method 23) and 100% (method 50),

with an average of 45.19%; males percentage varied between 0% (methods 23 and 50) and 71.97% (method 20), with an average of 52.39%. Age intervals varied between a 7-year (methods 30 and 32) and a 98-year interval (method 13), with an average of a 25.7-year interval.

BD_Sample_SES: Samples' SES was most commonly described as "Low to middle" (in 24% of the methods), followed by "Low" (in 12% of the methods), "Low to high" (in 8% of the methods) and "Middle to high" (in 4% of the methods). No sample was described as having an "High" SES. Half of the methods either did not report or did not have access to the sample's SES profile (Figure 3.1.2.B).

BD_Study_material: A total 44% of the methods used dry bone as study material, which was followed by CT scan and X-ray of the sample's specimens (20% of the methods, each), MRI (in 12% of the methods) and, lastly, data retrieved from human cadavers (in 4% of the methods) (Figure 3.1.2.C).

BD_Methodological_approach: Regarding methodological approach, most of the methods (70%) were based on different stages of the epiphyseal union, with the most common (26%) being the 3 stages of epiphyseal union, followed by the 5 stages (20%) and 8 stages (8%) of epiphyseal union, as described by Cardoso (2008a, 2008b), Kellinghaus et al. (2010) and Schmeling et al. (2004), respectively. A total of 12% of the methods were based on the morphology of different anatomical areas and 16% on measurements (mainly of the long bones). Only one method used a combination of the 3 stages of epiphyseal union and measurements of the calcaneus (method 9) (Figure 3.1.2.D and Figure 3.1.2E).

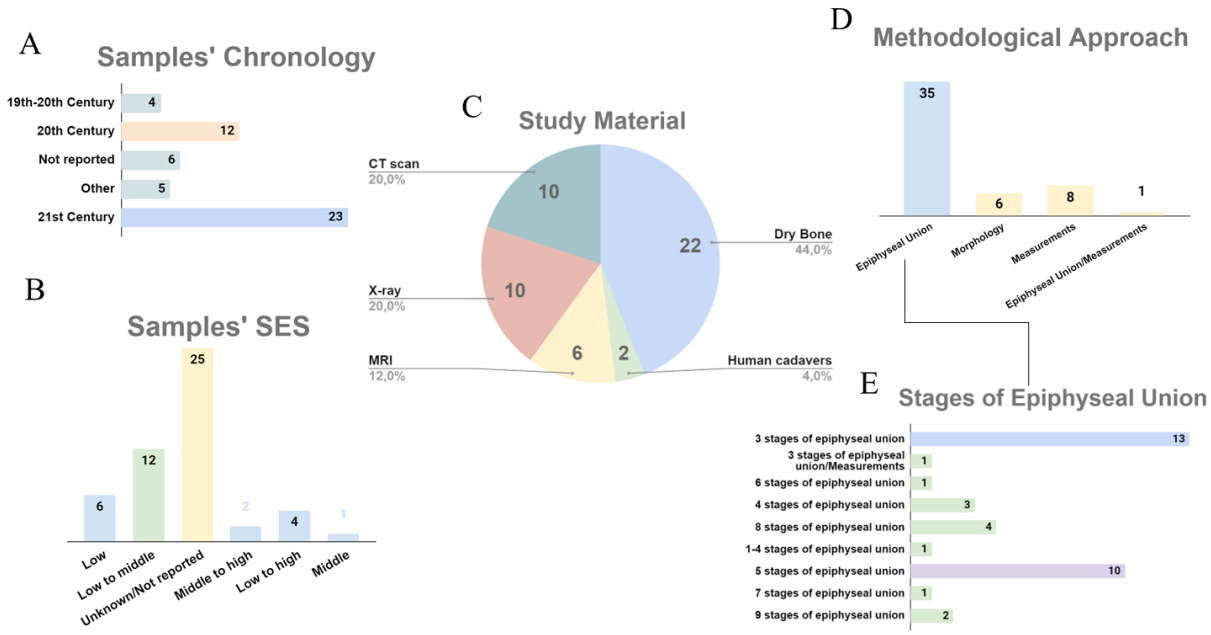

Figure 3.1.2.: *Charts regarding information about the BD methods used: A – bar chart of the samples' chronology; B – bar chart of the samples' SES; C – pie chart of the study materials used; D – bar chart of the methodological approach employed; E – bar chart regarding the stages of epiphyseal union used.*

BD_Anatomical_areas: More than half of the methods used anatomical regions from the appendicular skeleton: 34% were based on skeletal elements from the lower limb (most commonly the iliac crest and the proximal tibia), 24% were based on the upper limb (the most frequent anatomical areas being the medial extremity of the clavicle and the proximal humerus) and the last 10% was based on a combination of skeletal elements from the upper and the lower limb. A total of 24% of the BD methods were based on anatomical regions from the axial skeleton: 10% of which were based on the vertebral column (presacral and sacral vertebrae), 8% on the spheno-occipital synchondrosis, 4% on the sternum and only one method (method 27) was based on a combination of dental development, spheno-occipital synchondrosis and vertebrae (Figure 3.1.3.). Only 8% of the methods used a combination of skeletal elements from the axial and the appendicular skeleton. More than half of the methods (60%)

focused on a single skeletal element, while only 40% of the BD methods were based on a combination of anatomical areas (Figure 3.1.3.).

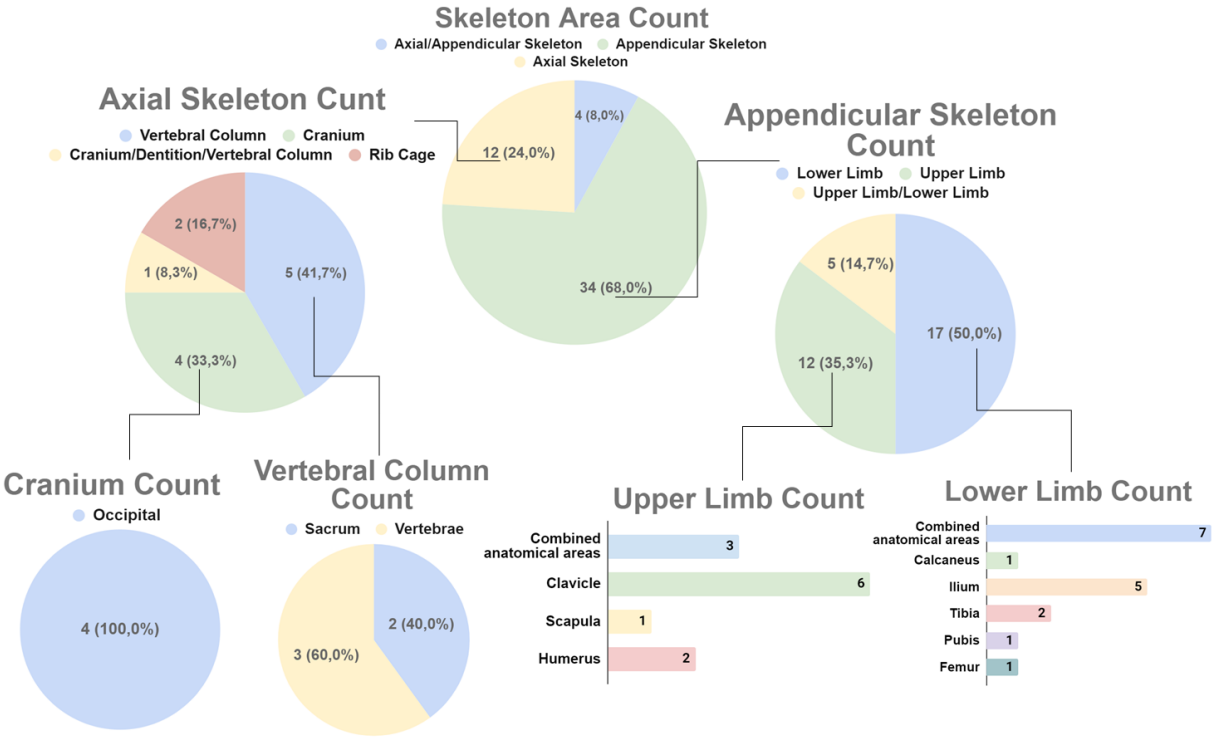

Figure 3.1.3.: *Pie charts and bar charts representing the frequency of each skeleton area used in the BD methods for AAD estimation.*

BD_Methods_performance: Only six of the total fifty BD methods used in the present dissertation presented values for accuracy of AAD estimation, meaning that only these methods used a separate sample to test the method created. These values ranged between 13% (a considerably low value) and 100%. The remaining methods' performance was evaluated through parameters such as: intra- and inter-observer agreement, r2, standard error of mean, correlation coefficient, coefficient of variation, sensitivity, specificity and inaccuracy (see Appendix A, Table A.1.).

DD_Methods: Regarding DD methods, the samples' most common geographical origin was North America (USA), followed by Europe (Britain and Spain) and Asia (India). Three of these samples were dated from the 20th Century and the remaining three were dated between the 18th and the 21st Century. Of the total of six samples used, three were reported as "Middle" SES, one as "Low to Middle" and the remaining three either did not report or access to this information was not available. DD methods were based on dental development and/or eruption. Four of these used X-ray as study material, one was based on a dry bone sample and method 54 was based both on dry bone and X-ray observations (see Appendix A, Table A.2.).

3.2. Total sample

The results of the overall sample (as described in section 2.2. Materials) were pooled to see which BD methods gave a similar AAD estimate to the reference methods - either DD or BD methods, depending on the degree of dentition preservation of each individual - used in each individual. A bar chart (Figure 3.1.1.) was plotted, allowing for a simpler comprehension of the results obtained. In Figure 3.2.1., it is visible that, for example, Coqueugniot and Weaver (2007) - method 1 - gave a similar AAD estimation to the reference methods in eight different individuals, while Cardoso and colleagues (2013b) - method 14 - only gave a similar AAD estimation to the reference methods in one individual. The methods were divided into three groups:

1) "Group 1" – includes BD methods that gave a similar AAD estimate to the reference methods in 8 different individuals (see Appendix A, Table A.3 for correspondence between methods codes and their authors and year of publication); this group includes a total of 6 methods, represented in the bar graph by the blue bars,
2) the group of BD methods that gave a similar estimate to the reference methods in 7 different individuals (here on "Group_2", see Appendix A, Table A.5 for a correspondence between methods codes and their authors and year of publication), this group includes a total of 11 methods, represented in the bar graph by the green bars,
3) and the group of BD methods that did this in six or less different individuals (represented by the bars in yellow). Only the information from the first two groups was considered, to compare the methods' variables.

Figure 3.2.1.: *Bar graph representing the number of times each BD methods gave a similar AAD estimate to the reference methods, in the total sample. Bars in light blue represent the BD methods that gave a similar AAD estimate to the reference methods in eight different individuals, the light green bars represent the BD methods that did this in seven individuals, and the light-yellow bars represent the methods that did this in six or less individuals.*

Group 1_Samples: Every BD method in Group_1 used samples from Europe, with half originating from Portugal (Figures 3.2.1.A and 3.2.1.B). These samples were dated between the 19th and the 21st

centuries and were linked to documented collections. Half of the methods used samples with either "Low" or "Low to middle" SES (the remaining half either did not report or did not have access to information on samples' SES) (Figure 3.2.1.C and 3.2.1.D).

Group 1_ Study_materials: More than half of these methods used dry bone samples, with only one using X-ray and another CT-scan to evaluate bone maturation (Figure 3.2.2.B).

Group1_Methodological_approach: The most common methodology employed was the evaluation of epiphyseal union stages, with a main focus on the methodologies described by Cardoso (2008a, 2008b), Kellinghaus et al. (2010) and Schmeling et al. (2004) of the three, five and eight stages of epiphyseal union, respectively. Only one method from the Group_1 used morphology of a skeletal element to evaluate bone development (Figures 3.2.2.C).

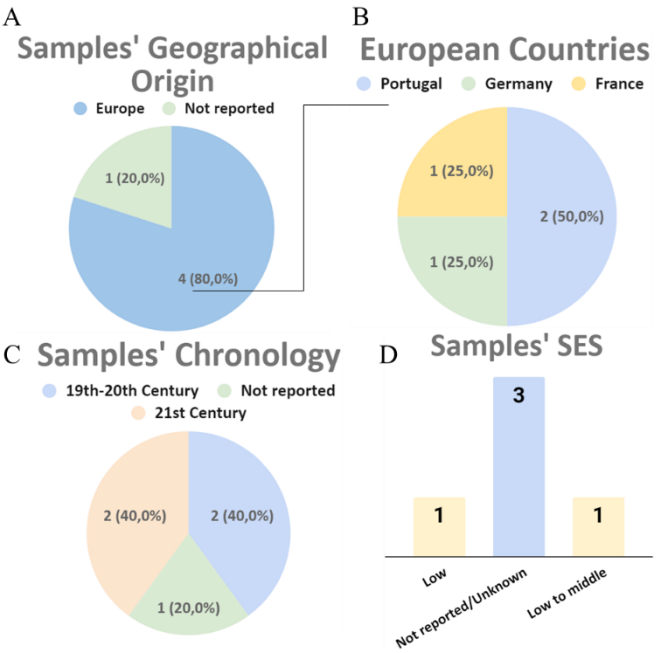

Figure 3.2.2.: *Pie charts and bar graphs of the BD methods in Group_1: A – Pie chart of the samples' geographical origins; B – Pie chart of frequency of European countries; C – Pie chart of samples' chronology; D – Bar graph on samples' SES.*

Group1_Anatomical_areas: In Group_1, only one method used an anatomical area from the axial skeleton (method 28, based on the morphology of the sternum). Two methods used a combination of skeletal elements from the axial and appendicular skeleton, and the remaining two were based on the Appendicular Skeleton: methods 16 and 42, based on the epiphyseal union of the iliac crest and the femur, respectively (Figure 3.2.2.A).

Group 1_Methods_performance: The smaller age range provided by Group 1 was in individuals [1296] and [1313] – method 5, with an age estimate of 22 years (1-year interval) – as well as in

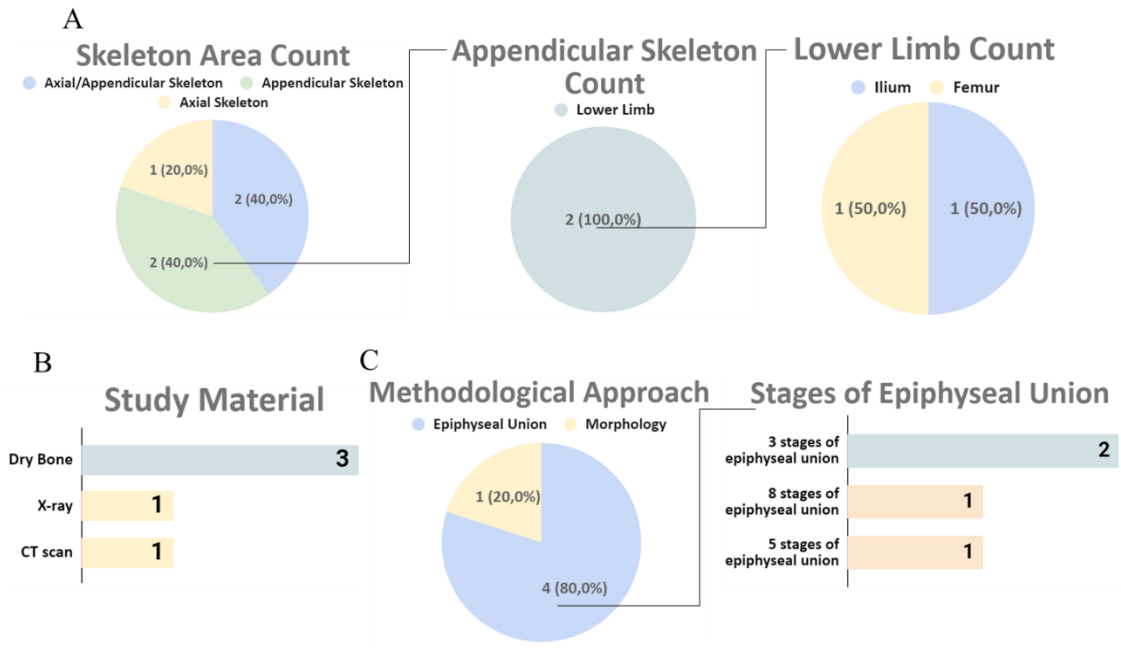

Figure 3.2.3.: *Pie charts and bar graphs of BD methods from Group_1: A – Pie charts representing the frequency of each skeleton area used in the BD methods for AAD estimation; B – Bar graph of the study material used; C – pie chart of the methodological approach employed and bar graph regarding the stages of epiphyseal union used.*

individuals [1406] and [1429] – , with an age estimate of 25 years (1-year interval). The larger age range was given by method 28 in individual [575] – 0-20 years (21-year interval). The lowest age interval average (in years) was 5.3 years (method 5) and the highest was 18.9 years (method 16) (see Appendix A, Table A.4).

Group 2_Samples: Group 2 comprehended a higher number of BD methods, introducing more variety to these results. In this group, most of the samples still originated from Europe (more commonly from Portugal), but a considerable percentage of the samples also stemmed from Asia (Turkey and Japan) and Oceania (Australia) (Figure 3.2.4.). The samples from Group_2 were dated between the 20th and the 21st Century (with >50% being from the current century), and SES status encompassed classification such as "Low", "Middle to high" and "Low to middle" (the latter being the most frequent). In more than half of these samples, SES was either not reported or not known (Figures 3.2.5.B and 3.2.5.C).

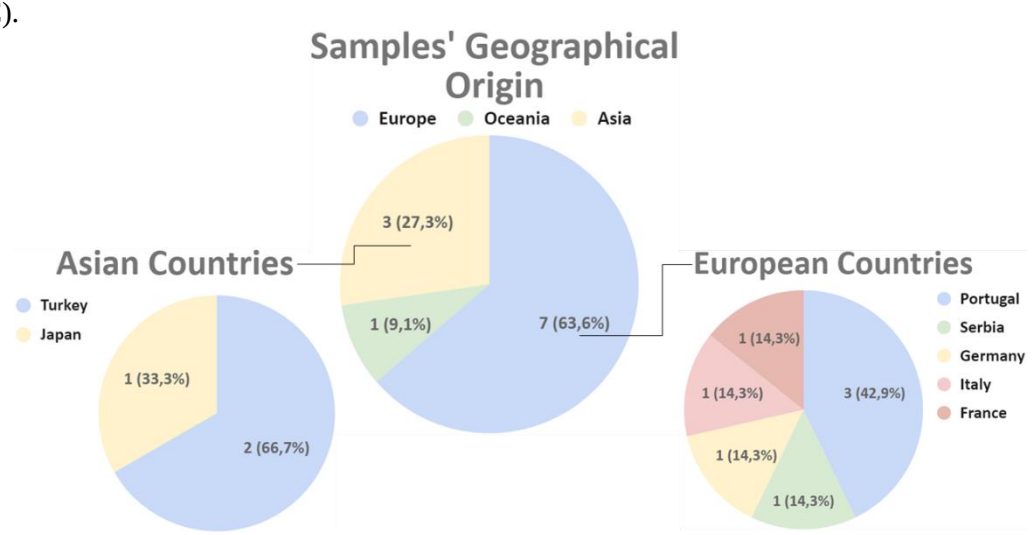

Figure 3.2.4.: *Pie charts of BD methods in Group_2: the centre pie chart represents the frequency of each samples' geographical origin, and the lower two pie charts represent the frequency of each country of origin from Asia and Europe.*

Group2_Study_material: Dry bone, MRI and CT scan samples were equally frequently used, with one method also using a sample of human cadavers and another one using X-rays (Figure 3.2.5.A).

Group2_Methodological_approach: Only one method (method 17) used the morphology of a skeletal element to evaluate bone development. The remaining methods used different sets of stages of epiphyseal union, with the most frequent being the three and five stages, as described by Cardoso (2008a, 2008b) and Kellinghaus et al. (2010), respectively (Figure 3.2.5.D and 3.2.5. E).

Group2_Anatomical_Areas: only one method used a skeletal element from the axial skeleton (method 17, based on the epiphyseal union of the sacrum), while the remaining used anatomical areas from the appendicular skeleton: one used a combination of skeletal elements from the upper and lower limb, four used the upper limb (more commonly the epiphyseal fusion of the medial clavicle) and the other half was based on the lower limb (with one using the epiphyseal fusion of the proximal tibia, another the fusion of the iliac crest, and the remaining three used a combination of different anatomical areas) (Figure 3.2.6.).

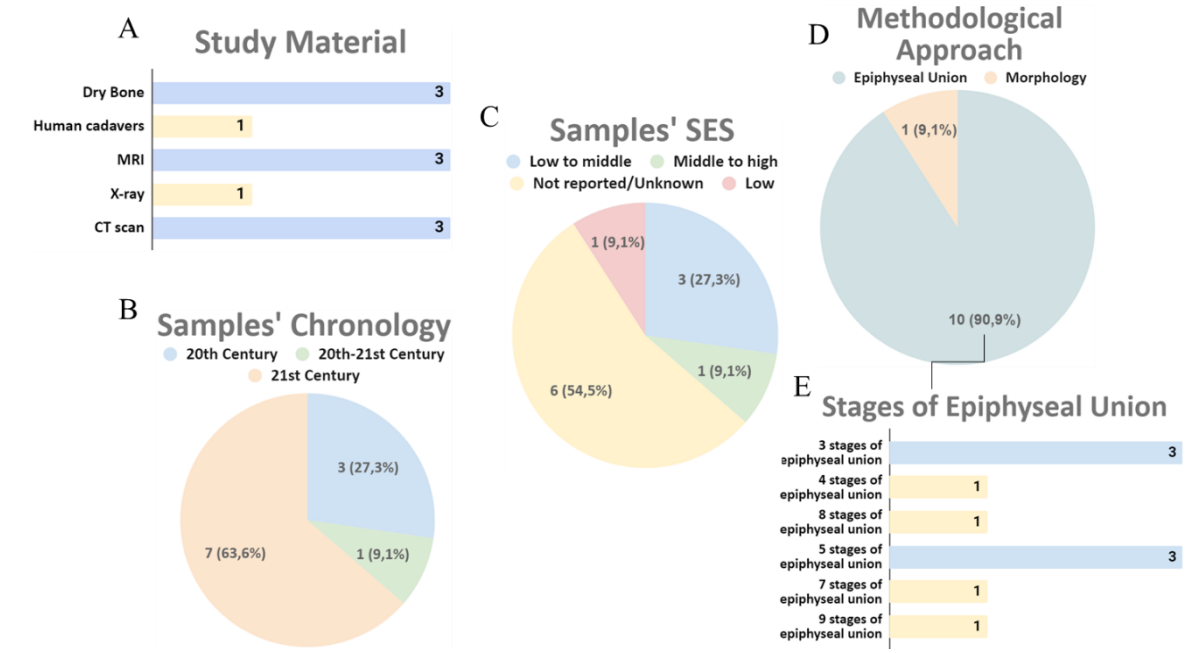

Figure 3.2.6.: *Charts regarding information about the BD methods used in Group_2: A – bar chart of the study material used; B – pie chart of the samples' chronology; C – pie chart of the samples' SES; D – pie chart of the methodological approach employed; E – bar chart regarding the different stages of epiphyseal union used.*

Group 2_Methods_performance: The smaller age range provided by this group of methods was in individual [1419] – method 26, with an age range of 18-20 years (3-year interval). The larger age range was given by method 11 in individual [575] – 0-22 years (23-year interval). The lowest age interval average (in years) was 7.7 years (method 2) and the highest was 14,6 years (method 8) (See Appendix A, Table A.6).

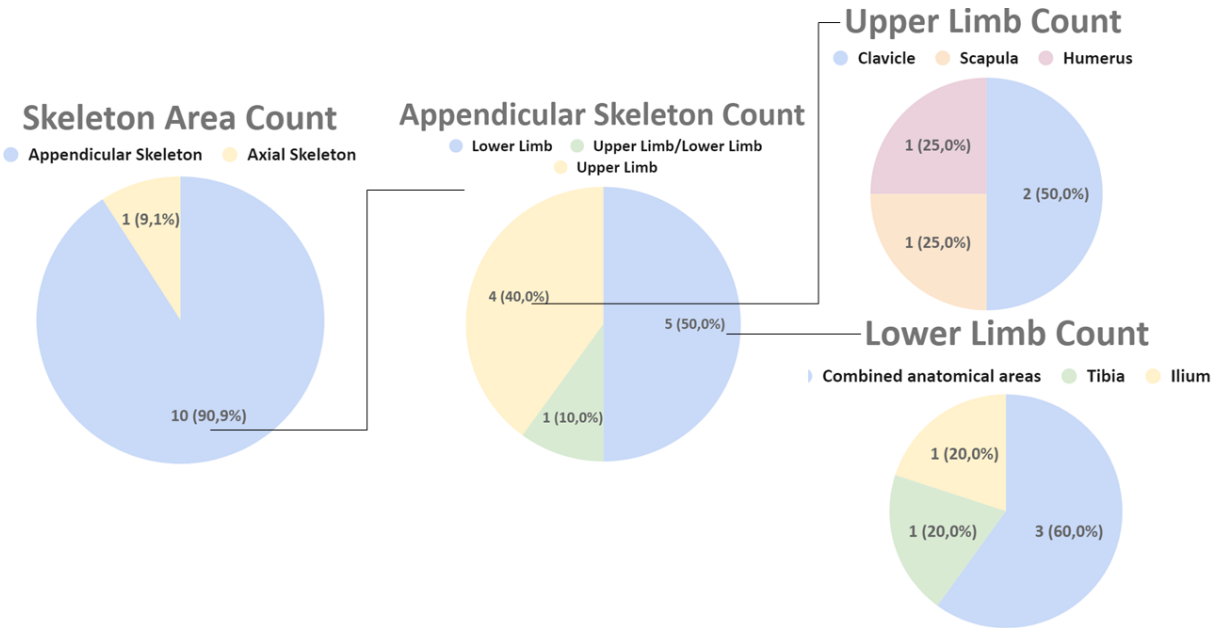

Figure 3.2.5.: *Pie charts representing the frequency of each skeleton area used in Group_2.*

Total sample_SIC: One SIC was observed in every individual from the total sample: changes to the long bones' diaphysis surfaces, here referred to as periosteal reactions (PR) (see section 1.5. on what these entail) of various degree (woven and lamellar bone were observed). These had a higher incidence on the long bones' diaphysis, especially in the lower limbs, but were also found in bones such as the sacrum and the os coxae in a smaller number of individuals (more details on these in the individual analysis - from subchapter 3.7. to 3.17.). Linear enamel hypoplasias (LEH) were found in every

individual with dentition preserved, with higher incidence on lower and upper incisors and canines, and molars presenting a smaller rate of LEH (the observation of LEH in the molars may have also been impaired by the high degree of dental wear observed in the older individuals from the sample). Finally, 45% of the individuals presented active *cribra femoralis* (CF) and only two individuals showed signs of active *cribra humeralis* (CH). No individual showed active occurrence of *cribra orbitalia* (CO), and only one skeleton had lesions suggestive of inactive periosteal reaction (PH) (Table 3.2.1).

Every individual analysed exhibited SIC. In some cases, more than one type of SIC was observed. This may be interpreted as suggestive of physiological stress during life, not limited to one episode but in some cases to chronic events. The extent of these periods of physiological stress may have varied, as well as the different individuals' ability to sustain stressful conditions. The number of SIC in the sample varied between one (in [872] and [1313]) and four (in [1214]) per individual. However, it is important to highlight that the individuals with the lower number of SIC were also the individuals with lower rates of preservation and completion - this means that the frequency of bone changes observed may be under represented, since preservation and conservation issues will always be an important factor to consider when analysing human remains (Table 3.2.1).

Table 3.2.1.: *Table representing the number of SIC present in every individual of the sample:*

EU	Enamel Hypoplasia	Porotic Hyperostosis	Cribra Orbitalia	Cribra Femoralis	Cribra Humeralis	Periosteal Reaction
[573]		×	×	✓	×	✓
[575]	✓	×	×	×	✓	✓
[872]		×	×	×	×	✓
[1008]	✓	×	×	✓		✓
[1214]	✓	×	×	✓	✓	✓
[1296]	✓	×	×	✓	×	✓
[1313]		×	×	×		✓
[1314]	✓	×	×	×	×	✓
[1406]	✓	×	×	✓	×	✓
[1419]		✓	×	×	×	✓
[1429]	✓	×	×	×	×	✓

× – absence of SIC; ✓ – presence of SIC.

3.3. Group_Dentition

This section addressed the results based on the individuals that compose Group_Dentition, which include only individuals who had teeth preserved (Figure 2.2.1). As stated in the methodology section, these results will also be used as a control group for the individuals from the total sample without dentition preserved.

The group of individuals with dentition preserved includes [575], [1008], [1214], [1296], [1314], [1406] and [1429].

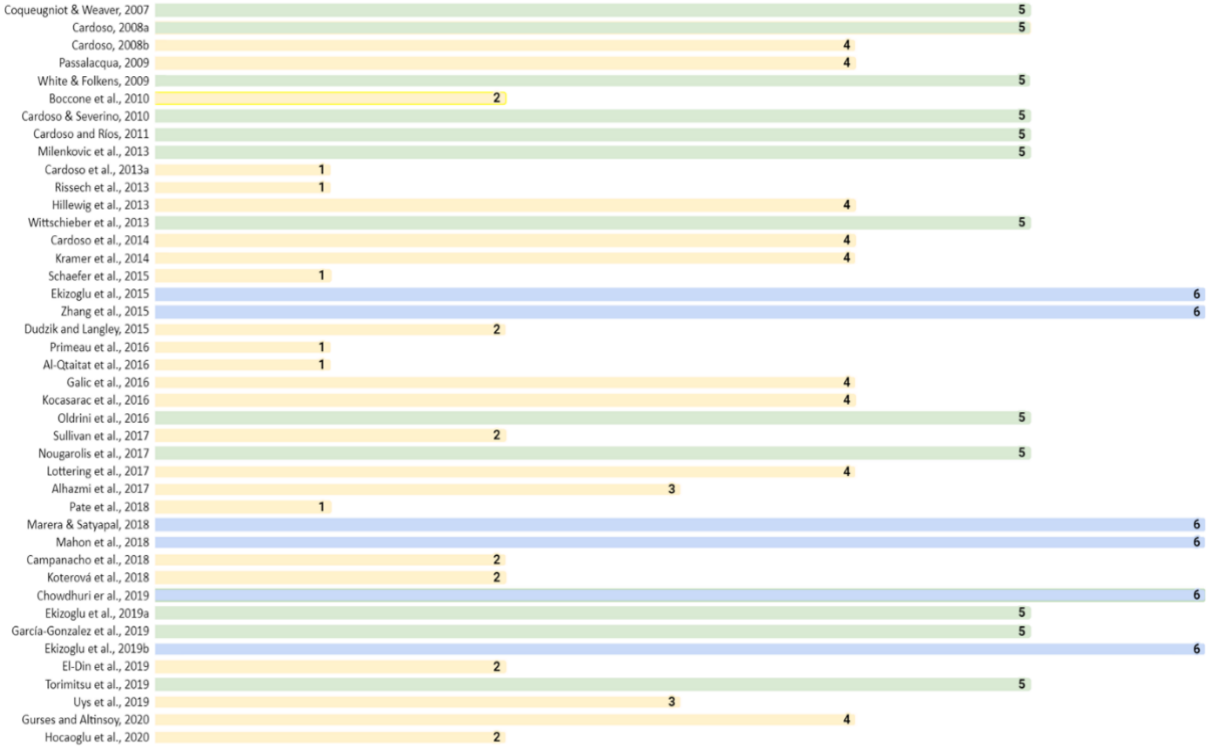

Figure 3.3.1.: *Bar graph representing the number of times each BD methods gave a similar AAD estimate to the DD methods, in individuals with dentition preserved. Bars in light blue represent the BD methods that gave a similar AAD estimate to the DD methods in six different individuals, the light green bars represent the BD methods that did this in five individuals, and the light-yellow bars represent the methods that did this in four or less individuals.*

The results obtained from each individual from Group_Dentition were pooled in a bar graph (Figure 3.3.1.), to see the number of times each BD method gave a similar AAD estimate to the DD methods. From the initial 50 BD methods, only 41 provided an AAD estimate similar to the DD methods in at least one individual. For example, and as observed in Figure 3.3.1., method 12 (Cardoso et al., 2013a) gave a similar AAD estimate to DD methods in only one individual - [1008] - while method 20 (Ekizoglu et al., 2015) did this in six different individuals - [575], [1214], [1296], [1314], [1406] and [1429].

This assessment allowed for the identification of two groups:

1) Group_Dentition1 - the group of BD methods that gave similar AAD estimates to DD methods in six different individuals each (Figure 3.3.1, in light blue);

2) Group_Dentition2 - the group of BD methods that gave a similar AAD estimate to DD methods in five different individuals each (Figure 3.3.1, in light green.

None of the BD methods represented in Figure 3.3.1. provided a similar AAD estimate to the DD methods in all of the seven individuals from Group_Dentition.

A total of six BD methods belong to Group_Dentition1: methods 20, 21, 36, 37, 40 and 43 (see Appendix A, Table A.8).

Group_Dentition1_Samples: These methods were based on samples from Asia (n=4: two from Turkey, and the remaining from China and India), and Africa (n=2) (Figure 3.3.2.A). These samples'

SES were described as “Low” (n=2), “Low to middle” (n=1) and “Low to high” (n=1). In the two remaining methods, the authors either did not report or did not know the samples’ SES (Figure 3.3.2.C). Chronologies ranged between the 20th (n=1) and the 21st Century (n=4) (Figure 3.3.2.B).

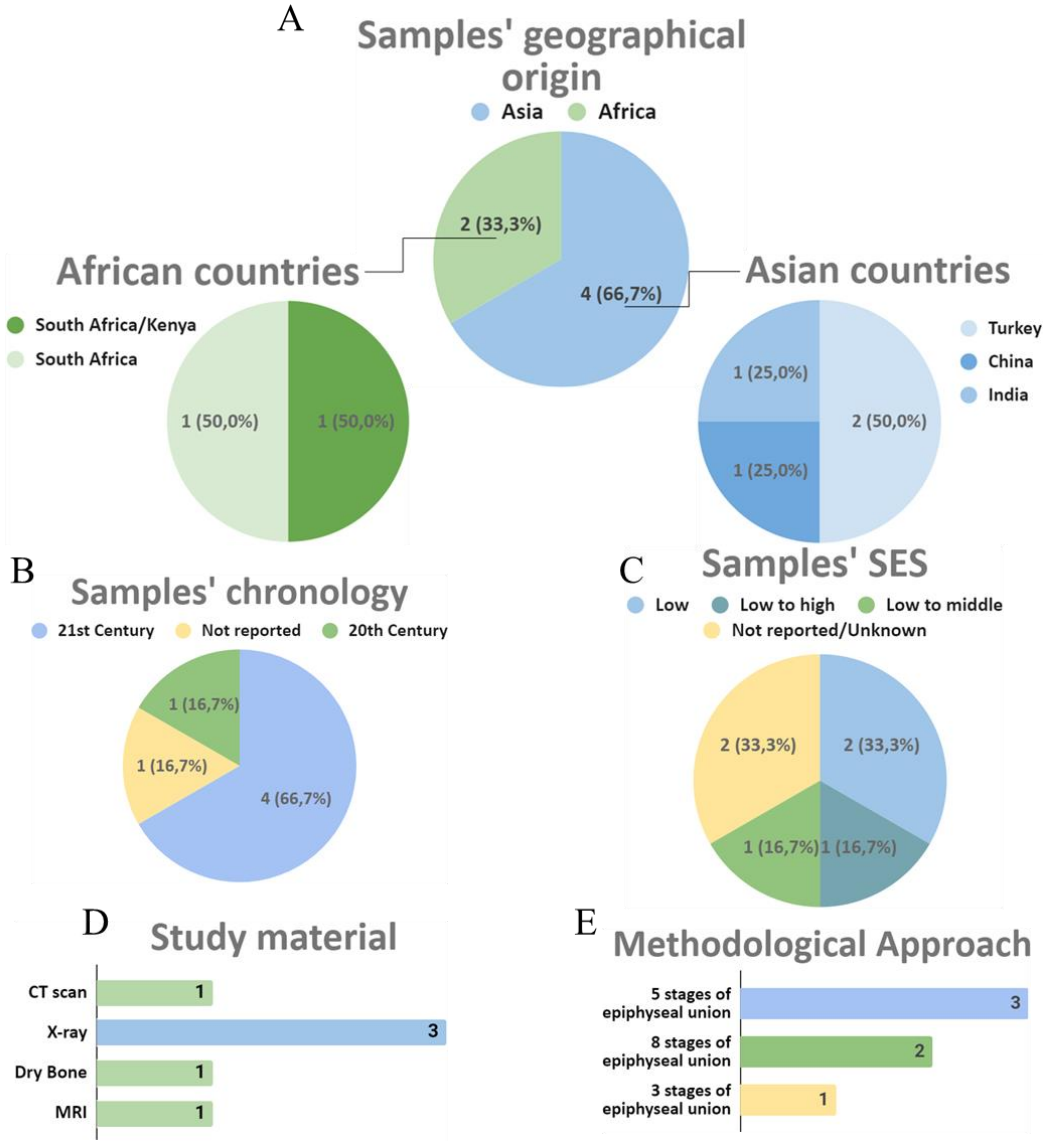

Figure 3.3.2.: *Pie charts and bar graphs of BD methods from Group_Dentition1: A – Pie charts representing the frequency of each BD methods’ samples geographical origin; B – pie chart of the samples’ chronology; C – pie chart of the samples’ SES; D – bar graph of the study materials used; E – bar graph of the methodological approach’s frequency in Group_Dentition1 (different stages of epiphyseal union).*

Group_Dentition1_Study_material: X-ray (n=3) was the most commonly used study material. The remaining three methods were developed based on dry bone, MRI and CT scan samples (Figure 3.3.2.D).

Group_Dentition1_Methodological_approach: All methods in Group_Dentition1 used methodological approaches based on different stages of epiphyseal union. The most common approach was the 5 stages (n=3), followed by the 8 stages (n=2) and 3 stages (n=1) of epiphyseal union (Figure 3.3.2.E).

Group_Dentition1_Anatomical_areas: All methods from Group_Dentition1 used anatomical areas from the Appendicular Skeleton (Figure 3.3.3.):

- four methods used skeletal elements from the upper limb (medial clavicle – n=3 - and proximal humerus – n=1));

- two methods used the lower limb (iliac crest - n=2).

All the methods from Group_Dentition1 focused on only one skeletal element, rather than combining different anatomical areas.

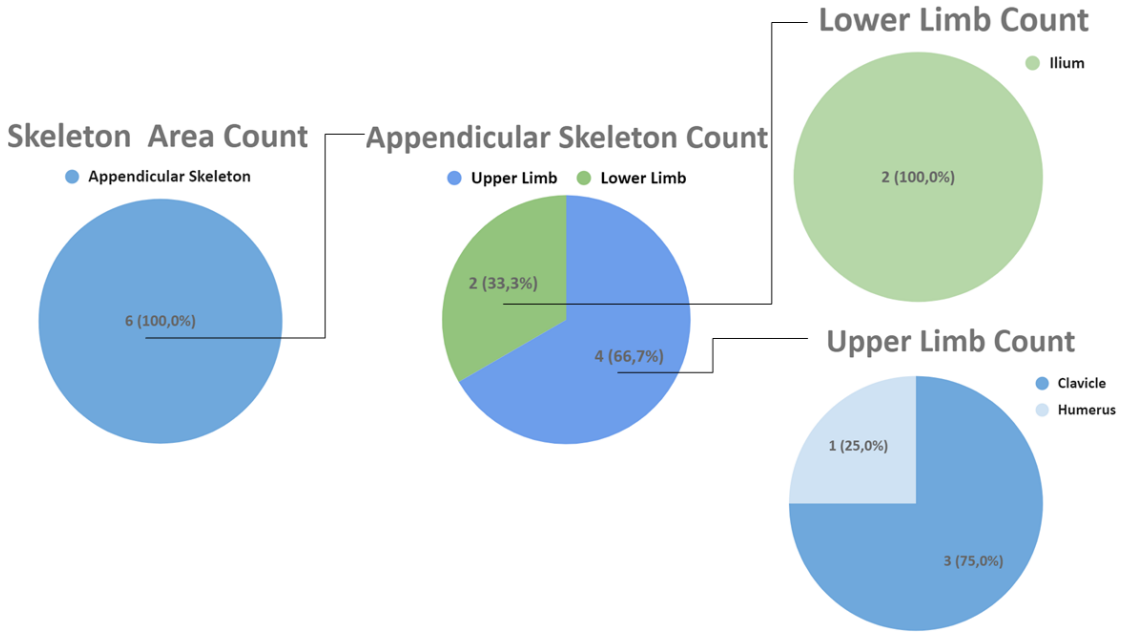

Figure 3.3.3.: *Pie charts representing the frequency of each skeleton area used in Group_Dentition1.*

Group_Dentition1_Age_ranges: The smaller age range provided by this group of methods was in individual [1429] – method 36, with an age range of 26-30 years (5-year interval). The larger age range was given by method 37 in individual [1214] – 13-30 years (18-year interval). The lowest age interval average (in years) was 8.3 years (method 21) and the highest was 14 years (method 37) (see Appendix A, Table A.7.).

Group_Dentition2 includes methods 1, 2, 5, 8, 10, 11, 16, 28 and 31 (see Appendix A, Table A.9 for a correspondence between methods codes and their authors and year of publication). Since this group included a higher number of BD methods, the results obtained also presented a higher range of characteristics, when compared to Group_Dentition1.

Group_Dentition2_Samples: All methods from Group_Dentition2 derive from Europe (n=8: four from Portugal, two from France, one from Serbia and one from Germany; Figure 3.3.4.A)- Samples' chronology ranged between the 19th and the 21st Century (n=3 from the 20th Century and n=3 from the 21st Century; Figure 3.3.4.C). Samples' SES were described as "Low" (n=1), "Low to middle" (n=3) and "Middle to high" (n=1), with the remaining three methods either not reporting or not having access to this information (Figure 3.3.4.D).

Group_Dentition2_Study_material: In the methods from Group_Dentition2, the most common study material was dry bone samples (n=5), with the remaining methods using either human cadavers (n=1), X-ray (n=1) or CT scans (n=2) (Figure 3.3.4.E).

Group_Dentition2_Methodological_approach: More than half of these used different stages of epiphyseal union as a methodological approach (n=7; Figure 3.3.4.F). The most common approaches were the 3 stages (n=5), followed by the 5 (n=1) and 8 (n=1) stages of epiphyseal union (Figure 3.3.4.G).

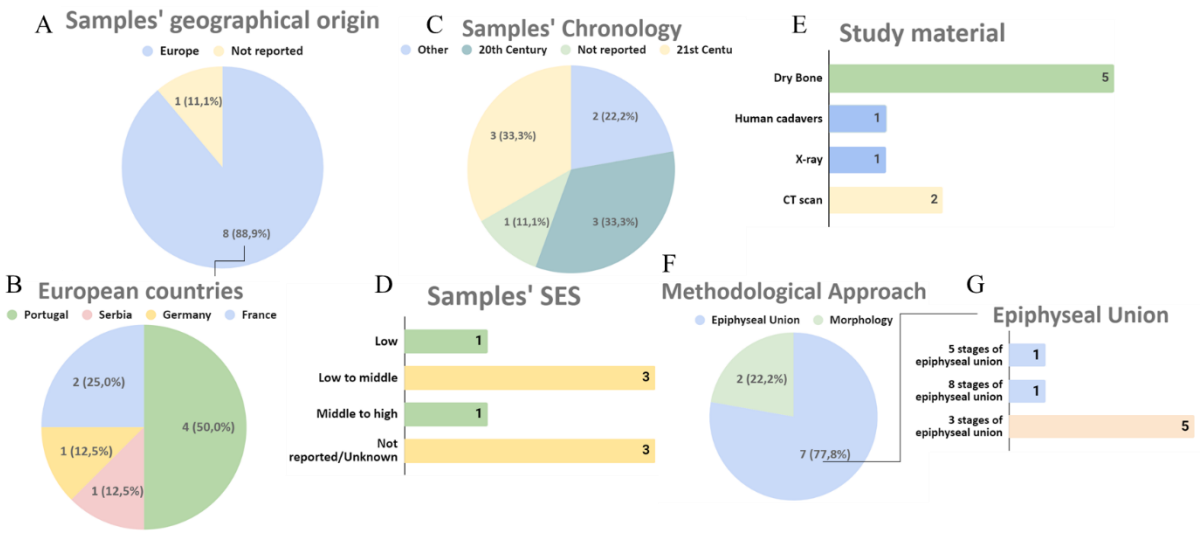

Figure 3.3.4.: *Pie charts and bar graphs of BD methods from Group_Dentition2: A – Pie charts representing the frequency of each BD methods' samples geographical origin; B – pie chart of European countries' frequencies; C – pie chart of the samples' chronology; D – bar graph of the samples' SES; E – bar graph of the study materials used; F – pie charts on methodological approach employed by the BD methods; G – bar chart of the stages of epiphyseal union used in the BD methods from Group_Dentition2.*

Group_Dentition2_Anatomical_areas: More than half of these methods (n=5) were based on skeletal elements from the appendicular skeleton: two methods used the upper limb (medial clavicle: n=1; scapula: n=1) and another two used the lower limb (iliac crest: n=1; combination of different anatomical areas: n=1). Only two methods used anatomical areas from the axial skeleton to estimate AAD (presacral vertebrae: n=1; sternum: n=1). Four methods from Group_Dentition2 used a combination of anatomical areas, while the remaining methods focused on only one skeletal element (Figure 3.3.5.).

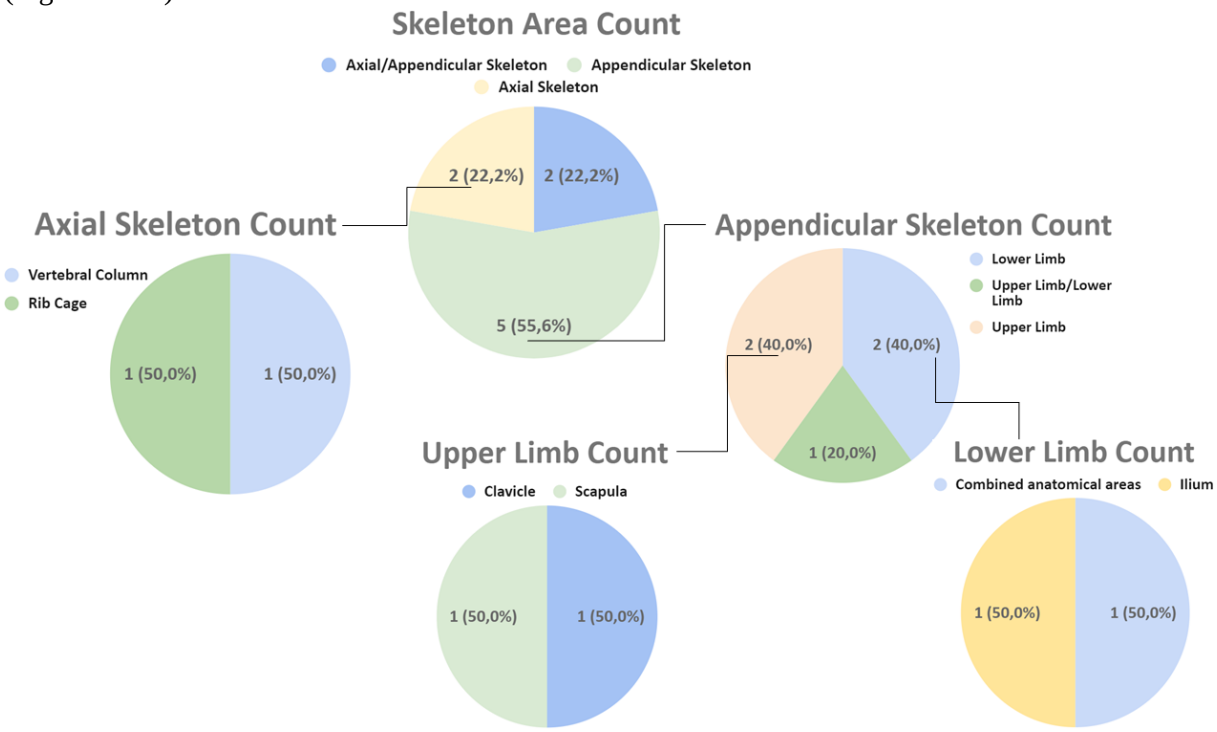

Figure 3.3.5.: *Pie charts representing the frequency of each skeleton area used in Group_Dentition2.*

Group_Dentition2_Age_ranges: Age ranges provided by the methods from Group_Dentition2 varied from a minimum of a 1-year interval in methods 5 and 28 (age estimate of 22 years in individual [1296], and of 25 years of age in individuals [1406] and [1429], respectively) and a maximum of a 23-

year interval, in method 11 (age estimate of 0-22 in individual [1214]. The lowest age interval average (in years) was 5.8 years (methods 1 and 5) and the highest was 14.8 years (method 8) (see Appendix A, Table A.10).

Group_Dentition2_Methods_accuracy: From these BD methods, only method 28 provided a value for accuracy, while the remaining methods' performance was evaluated through other parameters, such as intra- and inter-observer agreement, standard error of mean, correlation coefficient, etc. (for more details, see Appendix A, Tables A.9).

Group_Dentition_SIC: Regarding SIC frequency, the entire sample presented LEH and PR to different extents. More than half of the individuals from Group_Dentition (57%) presented CF; CH was also observed in two individuals. The number of SIC per individual ranges between two (in [1429] and [1314]) and four (in [1214]) (Table 3.3.1.).

Table 3.3.1.: *Table of SIC frequency in each set of human skeletal remains from the group of individuals with dentition preserved:*

EU	Enamel Hypoplasia	Porotic Hyperostosis	Cribra Orbitalia	Cribra Femoralis	Cribra Humeralis	Periosteal Reaction
[575]	✓	✗	✗	✗	✓	✓
[1008]	✓	✗	✗	✓		✓
[1214]	✓	✗	✗	✓	✓	✓
[1296]	✓	✗	✗	✓	✗	✓
[1314]	✓	✗	✗	✗	✗	✓
[1406]	✓	✗	✗	✓	✗	✓
[1429]	✓	✗	✗	✗	✗	✓

✗ *– absence of SIC;* ✓ *– presence of SIC.*

3.4. Group_No_Dentition

This section explores the results of individuals [573], [872], [1313] and [1419]. These are the ones with no dentition available. The results obtained from these individuals were pooled to see the number of times each BD method gave a similar AAD estimate to the reference BD methods (that correspond to Group_Dentition1 – the methods with more similar AAD estimates to the DD methods in the group of individuals with dentition preserved). Figure 3.4.1. Shows a bar plot representing these results; for example, method 1 (Coqueugniot & Weaver, 2007) gave a similar AAD estimate to the reference BD methods in three individuals - [573], [1313] and [1419] - while method 3 (Cardoso, 2008b) only did this in one individual - [1419]. From the total of 50 BD methods initially chosen, 6 were used as the reference methods. This means that only 44 methods were applied, from which only 39 gave a similar AAD estimate to the reference methods in at least one individual. One main group was obtained: the group of methods that gave a similar AAD estimate to the reference methods in three different individuals – here on, **Group_No_Dentition1** (Figure 3.4.1.).

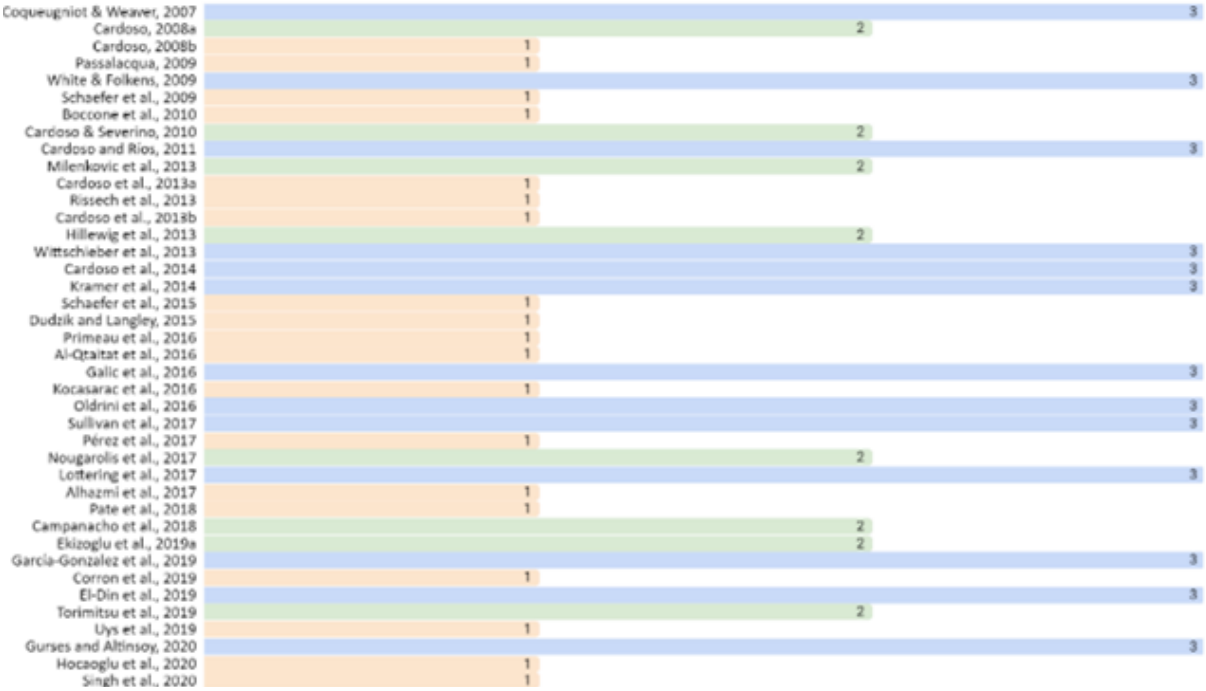

Figure 3.4.1.: *Bar graph representing the number of times each BD methods gave a similar AAD estimate to the BD reference methods, in individuals without dentition preserved. Bars in light blue represent the BD methods that gave a similar AAD estimate to the reference methods in three different individuals, the light green bars represent the BD methods that did this in two individuals, and the light-yellow bars represent the methods that did this in one individual.*

The remaining methods only gave an overlapping AAD estimate to the reference methods in a smaller number of individuals. None of the BD methods used comprised the total four individuals of Group_No_Dentition. The variables from the thirteen methods that compose Group_No_Dentition1 were compared.

Group_No_Dentition1 includes methods 1, 5, 10, 16, 17, 18, 26, 28, 29, 33, 42, 45 and 48 (see Appendix A, Table A.11 for a correspondence between methods codes and their authors and year of publication).

G**roup_No_Dentition1_Samples:** Eight of these methods used samples of European origin, half of these from Portugal (n=4). The remaining methods used samples from Asia (n=1), Africa (n=1), and Oceania (n=2) (Figure 3.4.2.). Samples' chronology ranged between de 19th (n=2) and 21st Century, with more than half from the latter (n=8) (Figure 3.4.3.D). SES was described as "Low" (n=1) and "Low to middle" (n=3) for four of the methods' samples. The remaining eight samples' SES were either not reported or not known (Figure 3.4.3.B).

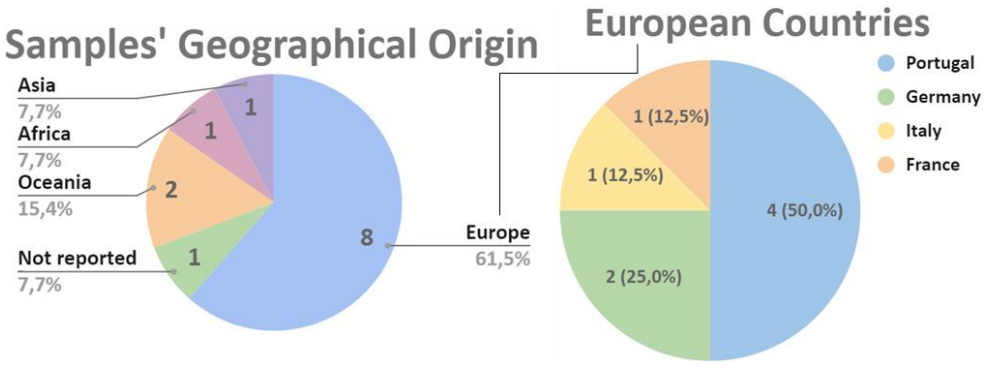

Figure 3.4.2.: *Pie charts representing the frequency of each BD methods' samples geographical origin.*

Group_No_Dentition1_Study_material: Dry bone was the most frequently used study material (n=5), followed by CT scan (n=3), MRI (n=3) and X-ray (n=2) (Figure 3.4.3.A).

Group_No_Dentition_Methodological_approach: Regarding methodological approach, only one method was based on morphology (methods 28 – based on sternum morphology). The remaining twelve methods used different stages of Epiphyseal union (Figure 3.4.3.C), with the most common approach being the 3 stages of epiphyseal union (n=5), followed by the 5 (n=2) and 8 (n=2) stages of epiphyseal union (as previously described) (Figure 3.4.3.E).

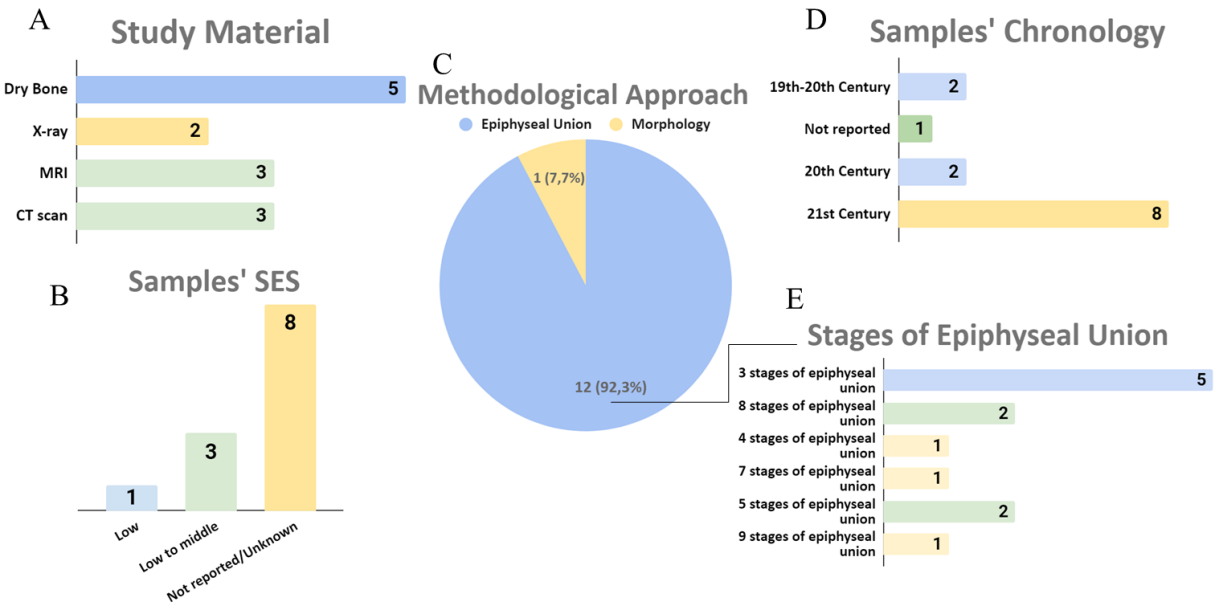

Figure 3.4.3.: *Pie charts and bar graphs representing the BD methods from Group_No_Dentition: A – bar graph of the study materials used; B – bar graphs of the samples' SES frequency; C – pie charts of the methodological approach employed by each method; D - bar graph of the samples' chronology; E – bar graph of the stages on epiphyseal union used.*

Group_No_Dentition1_Anatomical_areas: More than half (61,54%) of the methods focused on a single skeletal element, with the remaining methods using a combination of different skeletal elements.Anatomical areas from the appendicular skeleton were used in 61.5% of the BD methods, all of these from the lower limbs (iliac crest – methods 16 and 33; distal femur - method 42; proximal tibia – method 18 and 45; combination of skeletal elements of the lower limb – methods 26, 29 and 48). The remaining methods were based on the presacral vertebrae (method 10), sacrum (method17), sternum (method 28) and a combination of the spheno-occipital synchondrosis and the postcranial skeleton (methods 1 and 5) (Figure 3.4.4.).

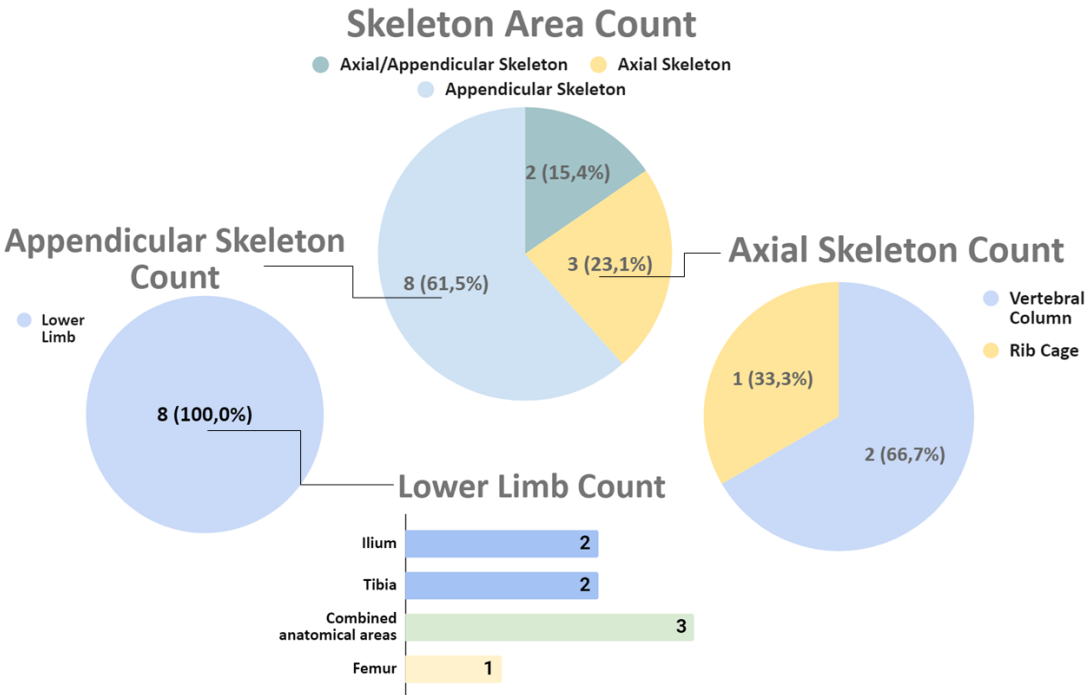

Figure 3.4.4.: *Pie charts representing the frequency of each skeleton area used in Group_No_Dentition.*

Group_No_Dentition1_Age_ranges: Age ranges provided by these methods varied from a minimum of a 1-year interval in method 5 (age estimate of 22 years, in individual [1313]) and a maximum of a 16-year interval in methods 18 (age estimate of 15-30 years, in individuals [573] and [1313]. The lowest age interval average (in years) was 4.3 years (method 5) and the highest was 15.7 years (method 18) (see Appendix A, Table A.12).

Goup_No_Dentition1_Accuracy: From the total of thirteen BD methods that constitute Group_No_Dentition, only two (methods 26 and 28) presented values for accuracy. The remaining methods' performance was evaluated through other parameters, such as intra- and inter-observer agreement values and other parameters (for more detailed information and values, see Appendix A, Table A.11).

Group_No_Dentition1_SIC: Regarding SIC frequency, the four individuals from this group presented PR. Only in one ([1419]) was observed a possible case of not-active PH, and in none of the four individuals was observed CO. CF was only registered in one individual ([573]) and CH was not observed in this sample. The minimum number of SIC presented in this sample was one (in [872] and [1313]) and the maximum number was two (in [1419]) (Table 3.4.1.). It is important to reinforce that these results may be skewed due to preservation and conservation rates, which may limit the number of SIC observed during the analysis.

Table 3.4.1.: *Table of SIC frequency in each set of human skeletal remains from the group of individuals without dentition preserved.*

EU	Porotic Hyperostosis	Cribra Orbitalia	Cribra Femoralis	Cribra Humeralis	Periosteal reaction
[573]	✗	✗	✓	✗	✓
[872]	✗	✗	✗	✗	✓
[1313]	✗	✗	✗		✓
[1419]	✓?	✗	✗	✗	✓

✗ – *absence of SIC;* ✓ – *presence of SIC.*

3.5. Individuals with partial epiphyseal fusion

Besides being divided into individuals with and without dentition preserved, the sample was also divided into individuals without epiphyseal union and individuals with partial epiphyseal union (Figure 2.2.1.). This division is justified by the fact that the AAD methods used for both groups are considerably different: while in the first group, AAD based on BD is mainly estimated by metric parameters, in the second group the degree of epiphyseal union mostly is used.

The group of individuals with partial epiphyseal union includes: [573], [1214], [1296], [1313], [1314], [1406], [1419] and [1429]. The results obtained from these individuals were pooled, to see the number of times each BD methods gave an overlapping AAD estimate to the reference methods used in each set of human skeletal remains. From the total of 50 BD methods initially chosen, only 39 gave a similar AAD estimate to the reference methods in at least one individual. Only one method (method 10 – Cardoso & Ríos, 2011) did this in every individual from this group. Method's 10 parameters were pooled with the parameters from the BD methods that gave a similar AAD estimate to the reference methods in seven different individuals from the present group, giving a total of thirteen different BD methods – here on, Group_Partial_Fusion (Figure 3.5.1.).

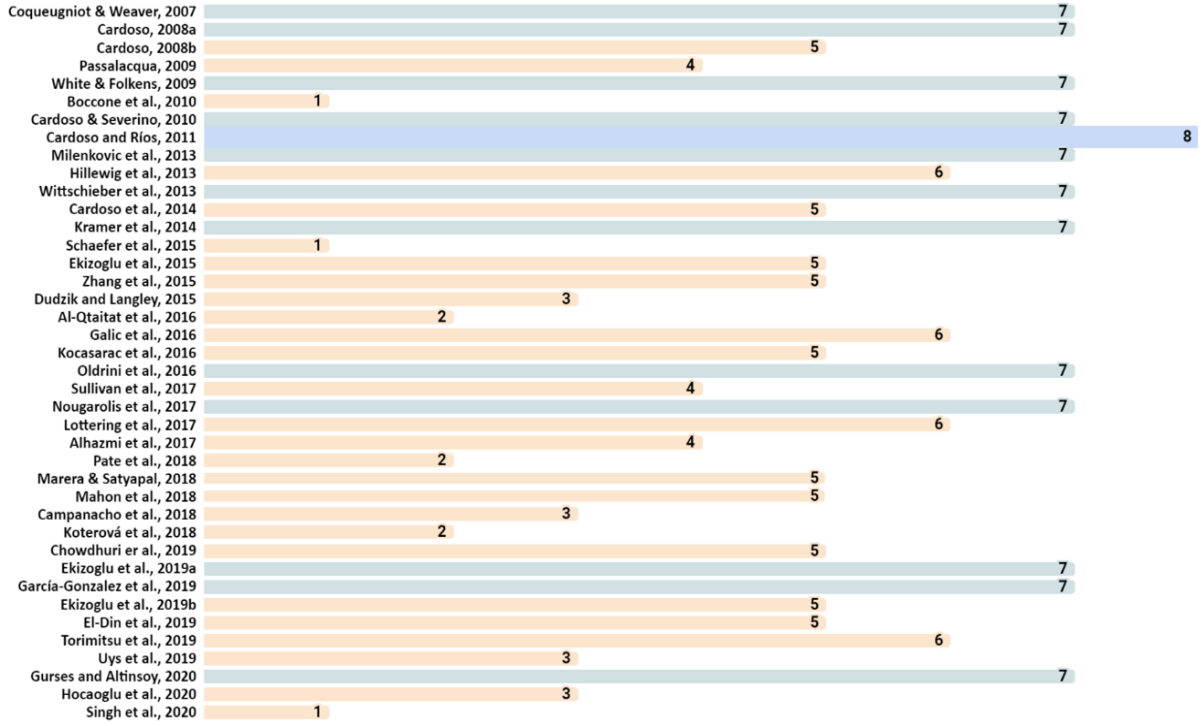

Figure 3.5.1.: *Bar graph representing the number of times each BD method gave a similar AAD estimate to the reference methods, in individuals with partial epiphyseal union. The bar in light blue represents the BD method that gave a similar AAD estimate to the reference methods in every individual from the sample, the light green bars represent the BD methods that did this in seven different individuals, and the light-yellow bars represent the methods that did this in six or less individuals.*

Group_Partial_Fusion includes methods 10, 1, 2, 5, 8, 11, 16 18, 28, 31, 41, 42 and 48 (see Appendix A, Table A.13 for a correspondence between methods codes and their authors and year of publication).

Group_Partial_Fusion_Samples: Ten of these methods had samples of European origin, of which half were from Portugal. Only two methods used Asian samples, more specifically from Turkey (Figure 3.5.2.). Samples' chronology ranged from the 19th (n=2) and 21st Century, with the latter being the most frequent (n=6) (Figure 3.5.3.C). Samples' SES was frequently described as "Low" (n=2) and "Low to middle" (n=4). Six methods either did not report or did not have access to the samples' SES (Figure 3.5.3.A).

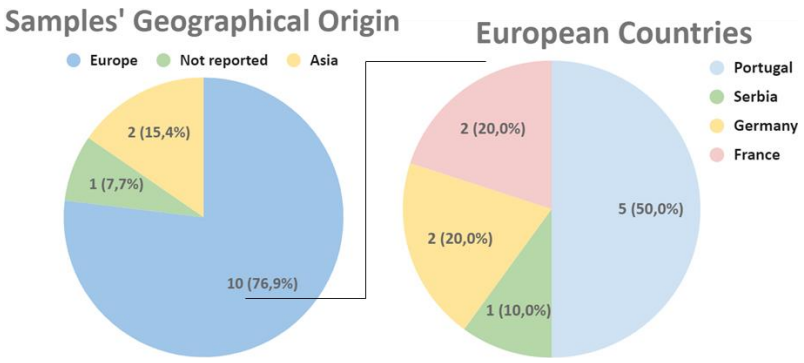

Figure 3.5.2.: *Scheme of the geographical distribution of the BD methods from Group_Partial_Fusion. The continental distribution of the samples is represented on the left pie chart, and the European countries distribution is represented on the right pie chart.*

Group_Partial_Fusion_Study_material: The most common study material used was dry bone samples (n=6), followed by MRI (n=3) and CT scan (n=2). The two remaining methods were based on X-ray (n=1) and human cadavers (n=1) (Figure 3.5.3.B).

Group_Partial_Fusion_Methodological_approach: Regarding methodological approach, 84.62% (n=11) of the methods used different stages of epiphyseal union, with the most common being the 3 stages (n=5), followed by the 5 stages (n=4) and 8 stages (n=2) of epiphyseal union. Only two methods were based on the morphology: one on the medial clavicle (method 11) and one on the sternum (method 28) (Figures 3.5.3.D and 3.5.3.E).

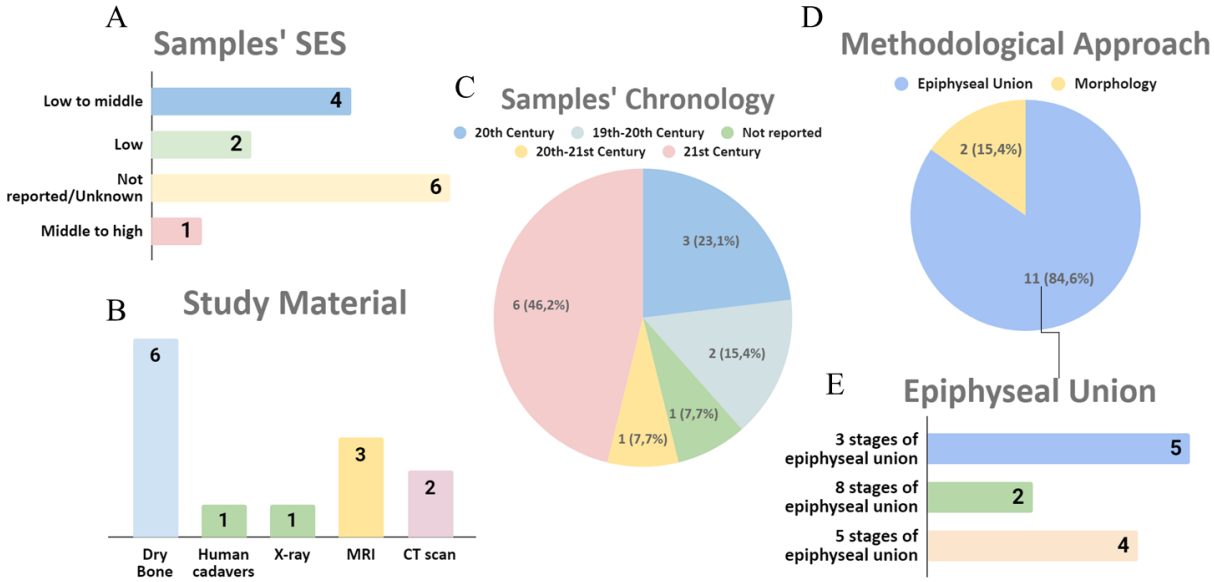

Figure 3.5.3.: *Charts regarding information about the BD methods used: A - bar chart of the samples' SES; B – bar graph of the study materials used; C – pie chart of the samples' chronology; D – pie chart of the methodological approach employed by the BD methods; E – bar chart regarding the stages of epiphyseal union used.*

Group_Partial_Fusion_Anatomical_areas: More than half of the methods (69.23%) used anatomical areas from the appendicular skeleton (Figure 3.5.4.): three methods the upper limb (medial clavicle – n=1; scapula – n=1; proximal humerus – n=1), five methods used the lower limb (iliac crest – n=1; distal femur – n=1; proximal tibia – n=1; combination of skeletal elements from the lower limb – n=2) and only one method used a combination of skeletal elements from the upper and lower limb. Two methods used anatomical areas from the axial skeleton (vertebrae – n=1; sternum – n=1) and another two a combination of anatomical areas from the axial and appendicular skeleton (Figure 3.5.4.). Only five of the methods used a combination of skeletal elements, while the remaining methods focused on only one.

Group_Partial_Fusion_Age_ranges: Age ranges provided by these methods varied from a minimum of a 1-year interval in methods 5 and 28 (age estimate of 22 years in individuals [1296] and [1313] and of 25 years in individuals [1406] and [1429], respectively) to a maximum of 23-year interval in method 11 (age estimate of 0-22 years in individual [1214]). The lowest age interval average (in years) was 4.6 years (method 28) and the highest was 15.1 years (method 42) (see Appendix A, Table A.14).

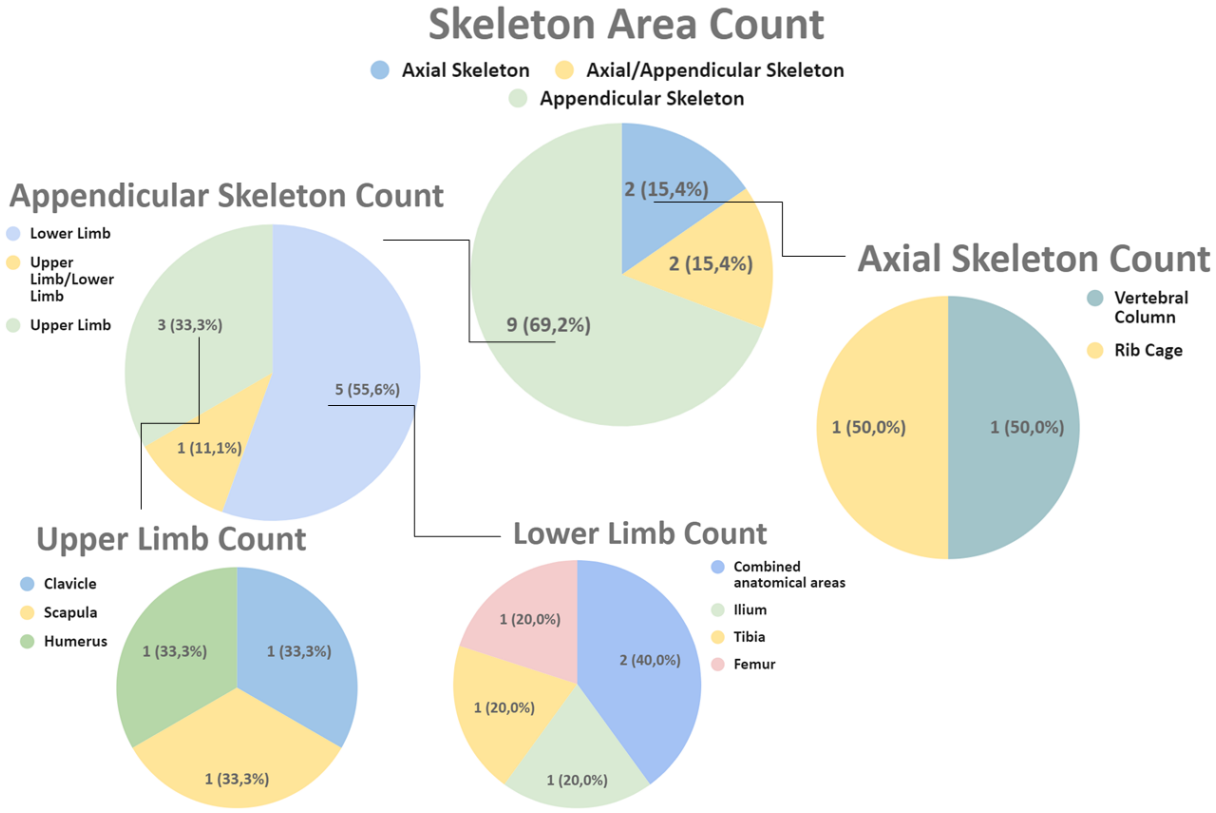

Figure 3.5.4.: *Pie charts and bar charts representing the frequency of each skeleton area used in the BD methods from Group_Partial_Fusion.*

Group_Partial_Fusion_Methods_accuracy: From the total 13 methods from Group_Partial_Fusion, only method 18 reported values for accuracy, while the remaining methods' performance was evaluated through parameters such as intra- and inter-observer agreement values, standard error of mean and coefficient of variation (for more detailed information and values, see Appendix A, Table A.13).

Group_Partial_Fusion_SIC: The most frequently observed SIC were PR and LEH (Table 3.5.1.). Both were present in every individual with partial epiphyseal union (LEH was not observed in the individuals for which dentition was not preserved and/or recovered). There was only one possible case of not active PH (in individual [1419]) and CO was not observed in the sample. CF was observed in four different individuals ([573], [1214], [1296] and [1406]), while CH was only registered for individual [1214].

Table 3.5.1.: *Table of SIC frequency in each set of human skeletal remains from the group of individuals with partial epiphyseal union.*

EU	Enamel Hypoplasia	Porotic Hyperostosis	Cribra Orbitalia	Cribra Femoralis	Cribra Humeralis	Periosteal Reaction
[573]		✗	✗	✓	✗	✓
[1214]	✓	✗	✗	✓	✓	✓
[1296]	✓	✗	✗	✓	✗	✓
[1313]		✗	✗	✗		✓
[1314]	✓	✗	✗	✗	✗	✓
[1406]	✓	✗	✗	✓	✗	✓
[1419]		✓?	✗	✗	✗	✓
[1429]	✓	✗	✗	✗	✗	✓

✗ *– absence of SIC;* ✓ *– presence of SIC.*

3.6. Individuals without epiphyseal fusion

The group of individuals without epiphyseal union includes [575], [872] and [1008]. The results obtained from these individuals were pooled to see the number of times each BD method gave an overlapping AAD estimate to the reference methods used in each set of human skeletal remains. From the total of 50 BD methods initially chosen, only 27 gave a similar AAD estimate to the reference methods in at least one individual. One main group was obtained: the group of methods that gave a similar AAD estimate to the reference methods in two different individuals – here on, Group_No_Fusion (Figure 3.6.1.). No methods gave a similar AAD estimate to the reference methods in the three individuals from this group simultaneously.

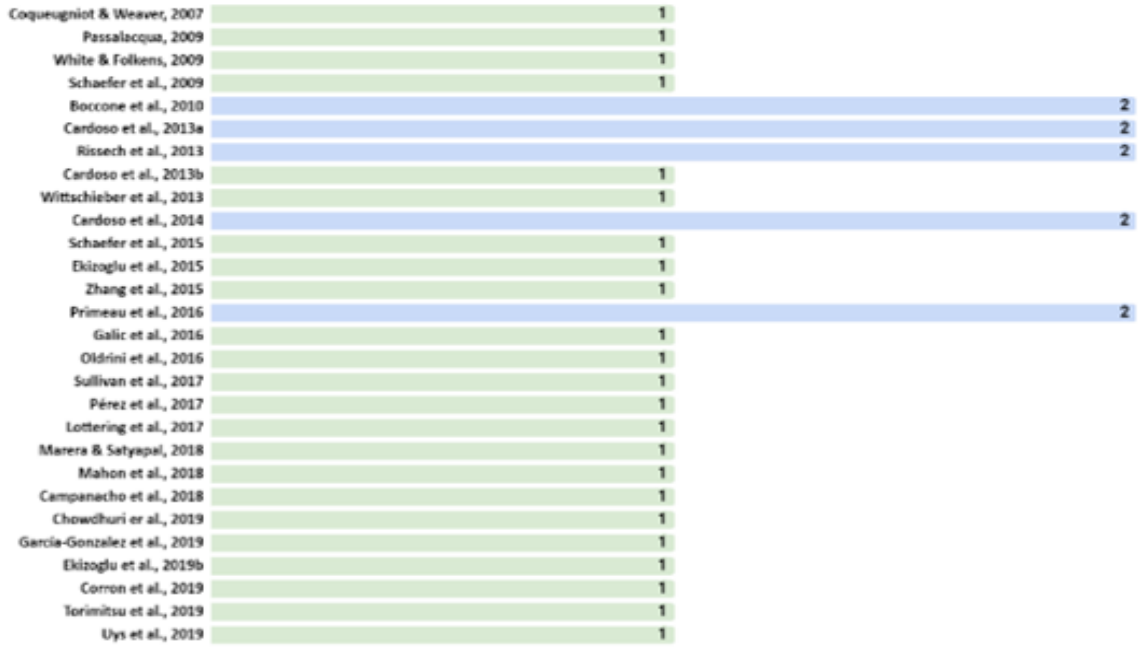

Figure 3.6.1.: *Bar graph representing the number of times each BD methods gave a similar AAD estimate to the refence methods, in individuals without epiphyseal union. Bars in light blue represent the BD methods that gave a similar AAD estimate to the DD methods in two different individuals, the light green bars represent the BD methods that did this in only one individual.*

Group_No_Fusion includes methods 7, 12, 13, 17 and 23 (see Appendix A, Table A.15 for a correspondence between methods codes and their authors and year of publication).

Group_No_Fusion_Samples: Four of the five methods' samples were European, of which half were from Portugal. A sample of African origin (Egypt) was used in only one case (Figure 3.6.2.). Samples' chronology ranged from 2300 - 1750 BCE (n=1) and the 20th Century, with the latter being the most frequent (n=2)) (Figure 3.6.3.A). Samples' SES was described either as "Low" (n=1) or as "Low to middle" (n=4) (Figure 3.6.3.C).

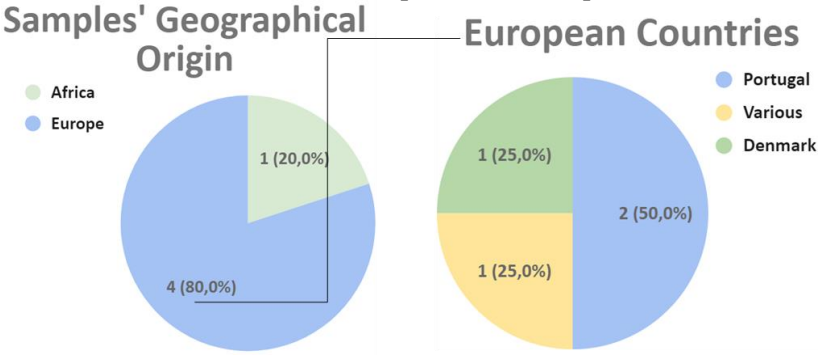

Figure 3.6.2.: *Scheme of the geographical distribution of the BD methods from Group_No_Fusion. The continental distribution of the samples is represented on the left pie chart, and the European countries distribution is represented on the right pie chart.*

Group_No_Fusion_Study_material: Dry bone samples were the most frequently used study material (n=4), and only one method used an X-ray sample (method 23) (Figure 3.6.3.D).

Group_No_Fusion_Methodological_approach: Regarding methodological approach, two methods were based on different stages of epiphyseal union, with one using the 4-stages (method 17) and the other the 6-stages of epiphyseal union (method 12). The remaining methods were based on measurements of various bones (n=3) (Figure 3.6.3.B).

Group_No_Fusion_Anatomical_areas: Four of the five methods used anatomical areas from the appendicular skeleton (Figure 3.6.3.E): one method used a combination of skeletal elements from the lower limb (ischiopubic ramus - method 12) and the remaining three used a combination of skeletal elements from the upper and lower limbs (methods 7, 13 and 23) (Figure 3.6.3.F). Only one method used an anatomical area from the axial skeleton (sacrum – method 17). Four of the methods were based on a combination of different skeletal elements, and only method 17 focused on a single bone: the sacrum.

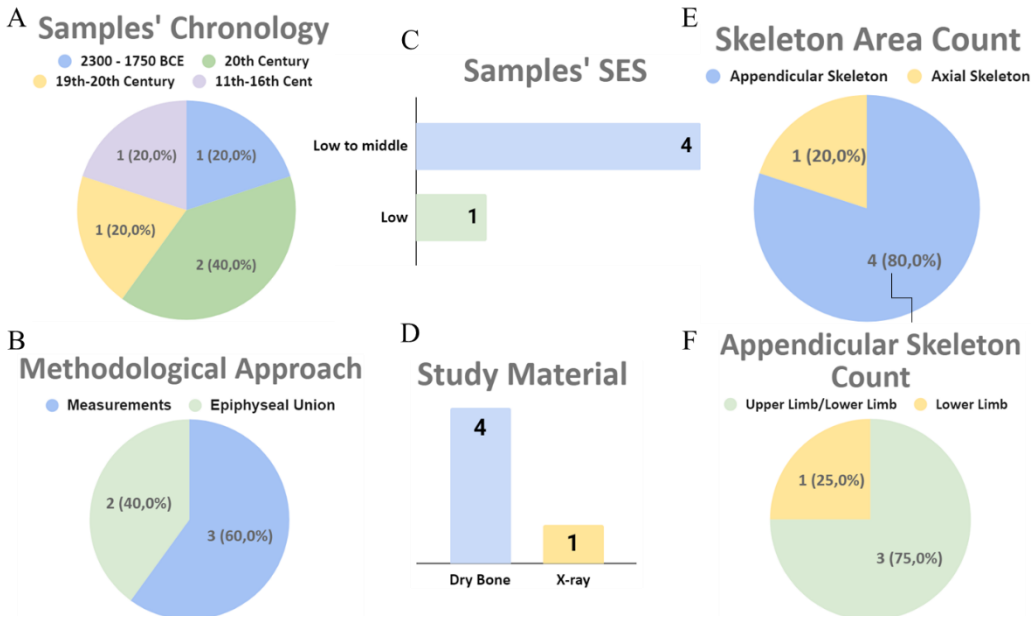

Figure 3.6.3.: *Charts regarding information about the BD methods used on Group_No_Fusion: A – pie chart of the samples' chronology; B – pie chart of the methodological approach employed by each of the BD methods; C – bar chart of the samples' SES; D – bar chart of the study materials used; E –pie chart regarding the skeleton area count; F – pie chart representing the Appendicular Skeleton Count.*

Group_No_Fusion_Age_ranges: Age ranges provided by these methods varied from a minimum of a 2-year interval in methods 7 and 12 (age estimate of 14.5-15.5 and 15-16.5years, respectively, in individual [575]) and a maximum of a 19-year interval, in method 17 (age estimate of 0-18 years in individual [575]). The lowest age interval average (in years) was 3 years (method 7) and the highest was 12 years (method 17). Method 12 gave the same age estimate to individuals [872] and [1008], being these the only individuals in which this method's AAD estimate coincided with the reference method used (see Appendix A, Table A.16).

Group_No_Fusion_Accuracy: From the total thirteen methods from Group_No_Fusion, there were no methods reporting values on accuracy. The methods only provided values for intra- and inter-observer agreement values and other parameters (for more detailed information and values, see Appendix A, Table A.15).

Group_No_Fusion_SIC: The most frequently observed SIC were PR and LEH (Table 3.6.1.). Both were present in every individual without epiphyseal union (LEH was not

Table 3.6.1.: *Table of SIC frequency in each set of human skeletal remains from the group of individuals without epiphyseal union.*

EU	Enamel Hypoplasia	Porotic Hyperostosis	Cribra Orbitalia	Cribra Femoralis	Cribra Humeralis	Periosteal reaction
[575]	✓	✗	✗	✗	✓	✓
[872]		✗	✗	✗	✗	✓
[1008]	✓	✗	✗	✓		✓

✗ – absence of SIC; ✓ – presence of SIC.

observed in individual [872], since there were teeth present). There were no cases of PH and CO. One individual presented CF and in another CH was observed. The minimum number of SIC in the individuals of this groups is one (in [872], in whom only PR was observed), and the maximum number was three (in individuals [575] and [1008].

3.7. [573]

After examining the sample as a whole and dividing it in four different groups, an individual-based analysis was performed to search for possible inconsistencies between AAD estimates provided by DD and BD, as well as between different anatomical areas (i.e.: axial vs. appendicular skeleton, upper vs. lower limbs, etc.). Hence, from this point forward, an individual analysis is presented.

For individual [573], an overall AAD estimate of 20-25 years was obtained based on the reference BD methods (as previously stated and explained, these methods correspond to Group_Dentition1 and were used as the reference methods in individuals without dentition preserved) (Figure 3.7.1.). Biological sex was estimated as female, based on the morphology of the *Os coxae*.

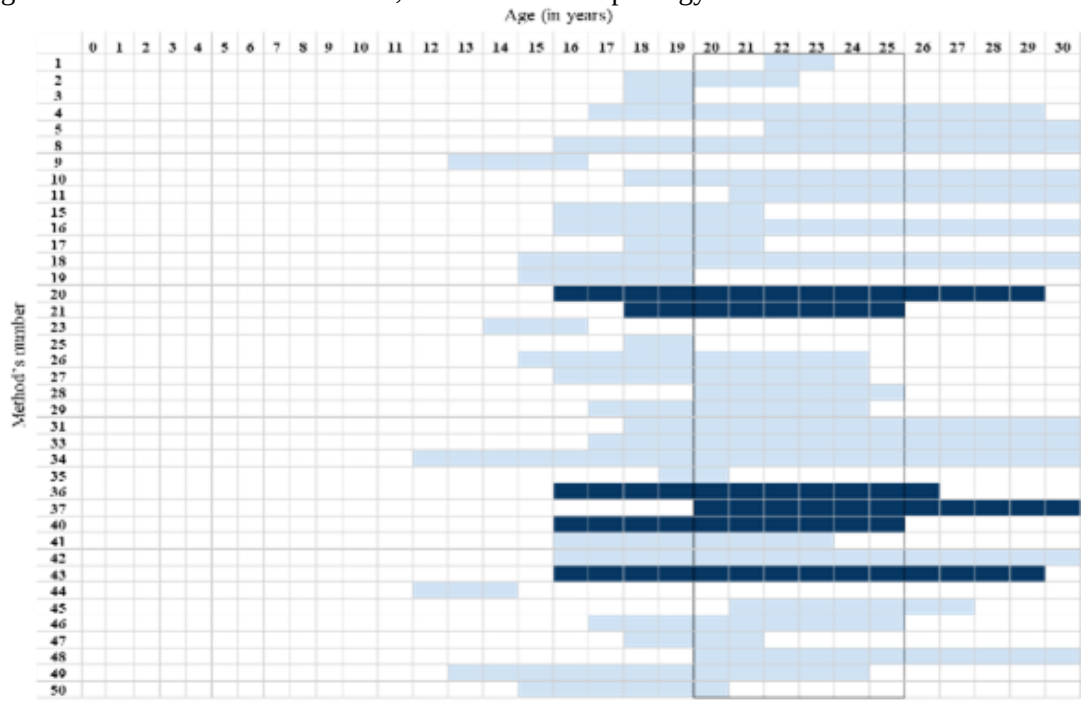

Figure 3.7.1.: *Diagram representing the AAD estimate provided by every method applied to individual [573]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis provides each method's number. The AAD estimate provided by the reference BD methods is represented by the bars in dark blue, and the bars in light blue represent the AAD estimate provided by the remaining BD methods. The black contour marks the age interval in which every reference BD method overlap.*

From the initial forty-four BD methods (the total fifty BD methods, minus the six BD methods used as reference in individuals without dentition – Chapter 3.3. for more details), only thirty-three could be applied to this set of human skeletal remains, either due to the overall stages of BD and/or rates of preservation and conservation of the human skeletal remnants. From these thirty-three methods, twenty-seven gave an AAD estimate that coincided (either partially or completely) with the estimate given by the reference BD methods, corresponding to a total of 81.8% of the methods applied (Figure 3.7.1.). Hereon, the group composed by these twenty-seven BD methods will be referred to as **Group_573**. Most of the remaining methods tended to underestimate age in relation to the reference BD methods. Age ranges provided by the methods from Group_573 varied between a 2-year (method 1 and 35) and an 18-year interval (method 34), with an average of a 9.67-year interval.

Group_573 includes methods 1, 2, 4, 5, 8, 10, 11, 15, 16, 17, 18, 26, 27, 28, 29, 31, 33, 34, 35, 41, 42, 45, 46, 47, 48, 49 and 50 (see Appendix A, Table A.17 for a correspondence between methods codes and their authors and year of publication).

Group_573_Samples: A total of 13 of these methods' samples are European, more frequently from Portugal (n=6), and 7 are Asian, with Turkey (n=4) being the most common country of origin of these samples (Figure 3.7.2.). Chronologies ranged from the 19th (n=2) to the 21st Century, with more than half (n=17) dated from the current century (Figure 3.7.3.B). SES was described as "Low" for three different samples, as "Low to middle" in five samples, "Low to high" in one sample, and "Middle to high" in two samples. The remaining 57.7% of the methods (n=15) either did not report or did not have access to each samples' SES (Figure 3.7.3.C).

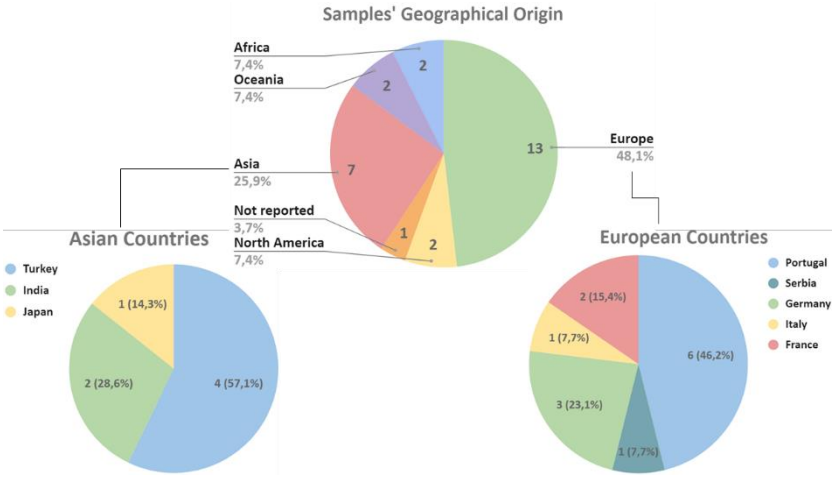

Figure 3.7.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_573. In the centre pie chart, the continent distribution is represented, followed by the pie charts of European and Asian countries (both countries with higher frequency in the centre pie chart).*

Group_573_Study materials: The most frequently used study materials were dry bone (n=8) and CT scan samples (n=8), followed by MRI (n=5), X-ray (n=4), and human cadavers (n=2) (Figure 3.7.3.A).

Group_573_Methodological_approachA total of 85.2% (n=23) of the methods from Group_573 used different stages of epiphyseal union to estimate AAD: the 3 stages of epiphyseal union were most frequently used (n=9), followed by the 5 (n=6) and 4 stages (n=3) of epiphyseal union (Figure 3.7.3.E). Four methods were based on the morphology of different skeletal elements, such as the sternum (method 28), the combination of spheno-occipital synchondrosis, dentition and vertebrae (method 27), the medial clavicle (method 11) and the sacrum (method 4) (Figure 3.7.3.D).

Group_573_Anatomical_areas: More than half (n=16) of the methods from group_573 used anatomical regions from the appendicular skeleton (Figure 3.7.4.): five methods were based on the upper limb (medial clavicle – n=3; proximal humerus – n=1; scapula – n=1); ten methods were based on the lower limb (iliac crest – n=2; distal femur – n=1; proximal tibia – n=2; combination of skeletal elements – n=5); one method was based on a combination of skeletal elements from the upper and lower limb (method 8). Nine methods were based on the axial skeleton (Figure 3.7.4.): two were based on anatomical areas from the cranium (spheno-occipital synchondrosis – n=2); five were based on skeletal elements from the vertebral column (sacrum – n=2; vertebrae – n=3); one method was based on the rib cage (sternum – n=1); one method was based on a combination of skeletal elements from the axial skeleton (spheno-occipital synchondrosis, dentition and vertebrae – n=1). Two methods were based on a combination of anatomical areas from the axial and appendicular skeleton (methods 1 and 5) (Figure 3.7.4.). A total of 33.33% (n=9) of the methods used a combination of skeletal elements, with the remaining methods (n=18) focusing on only one.

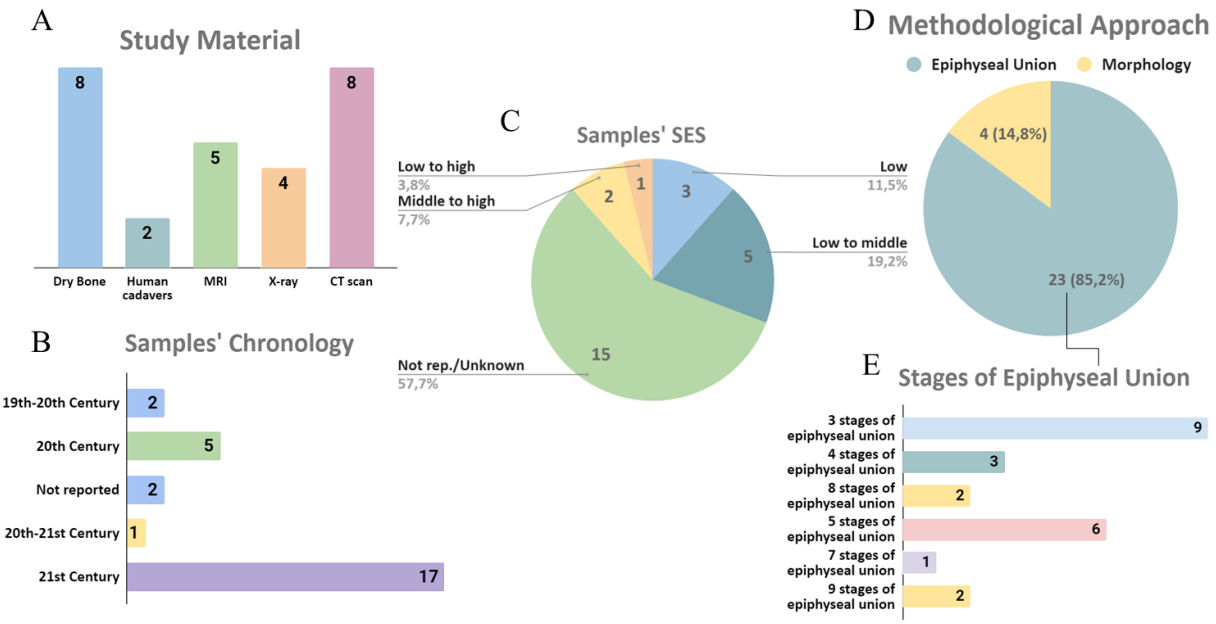

Figure 3.7.3.: *Charts regarding information about the BD methods from Group_573: A – bar chart of the study materials used; B – bar chart of the samples' chronology; C – pie chart of the samples' SES; D – pie chart of the methodological approach employed; E – bar chart regarding the stages of epiphyseal union used.*

Group_573_Accuracy: From the total twenty-seven methods from Group_573, only three methods reported values on accuracy (methods 4, 26 and 28). The other twenty-four methods only provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.17).

573_SIC: Individual [573] presented only two types of SIC visible: CF and PR. CF was observed bilaterally, although it could not be observed to its full extent in the left femur due to its preservation state. PR were observed in the long bones' diaphysis (tibia, femur, and humerus) through the presence of lamellar bone, as well as in the anterior surface of the sacrum, with a high degree of macroporosity visible.

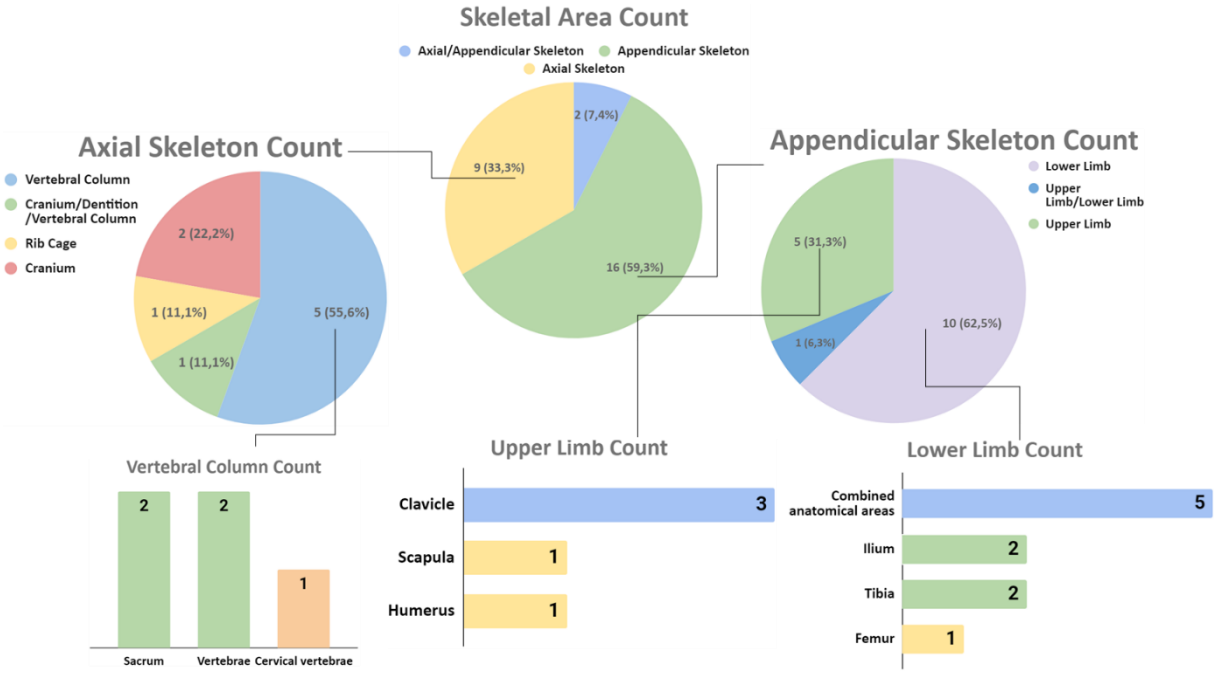

Figure 3.7.4.: *Pie charts and bar graphs representing the frequency of each skeleton area used in the BD methods from Group_573.*

3.8. [575]

For individual [575], an overall AAD estimate of 15.5 years was obtained based on the intersection of DD methods (Figure 3.8.1.). Biological sex was not estimated due to lack of fusion of the three bones from the *Os coxae*.

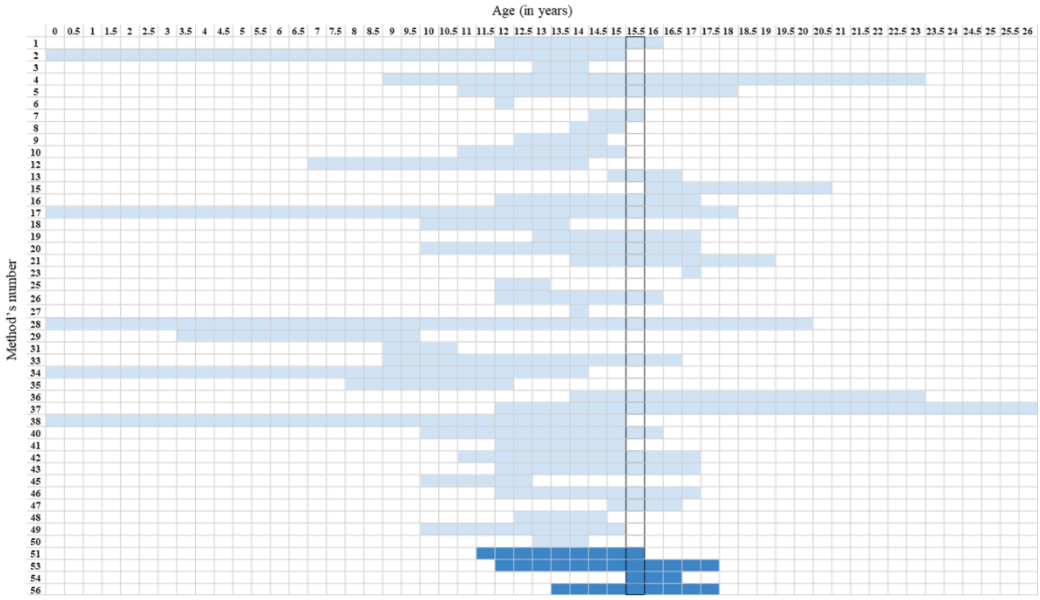

Figure 3.8.1.: *Diagram representing the AAD estimate provided by every method applied to individual [575]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which every DD method overlap.*

From the initial fifty BD methods, only forty-two could be applied, either due to the overall stages of BD and/or rates of preservation and conservation of the skeleton. From these forty-two methods, only twenty gave an AAD estimate that coincided (partially or completely) with the estimate given by the DD methods, corresponding to a total of 47.6% of the methods applied (Figure 3.8.1.). Hereon, the group composed by these twenty BD methods will be referred to as **Group_575**. Most of the remaining methods tended to underestimate age when compared to the DD methods. Age ranges of the methods from Group_575 varied between a 2-year (method 7, 13 and 47) and 21-year interval (method 28), with an average of an 8.15-year interval.

Group_575 includes methods 1, 4, 5, 7, 13, 16, 17, 19, 20, 21, 26, 28, 33, 36, 37, 40, 42, 43, 46, 47 (see Appendix A, Table A.18 for a correspondence between methods codes and their authors and year of publication).

Group_575_Samples: A total of 7 of these methods' sample are European, more frequently from Portugal (n=3); 5 are from Asia, mainly from Turkey (n=2); 4 of the remaining methods are African, either from South Africa (n=2), Egypt (n=1) and/or Kenia (n=1) (Figure 3.8.2.). Chronologies ranged between 2300-1750 BCE and the 21st Century, with half dated from the current century (Figure 3.8.3.C). SES was described either as "Low" (n=3), as "Low to high" (n=3) or as "Low to middle" (n=5). The remaining nine methods either did not report or did not have access to the respective samples' SES (Figure 3.8.3.B).

Group_575_Study_materials: The most frequently used study materials were dry bone samples (n=8), followed by X-ray (n=7), CT scan (n=4) and MRI samples (n=1) (Figure 3.8.3.A).

Group_575_Methodological approach: A total of 78.9% (n=15) of these methods used different stages of epiphyseal union to estimate AAD: the most frequent stages used were the three (n=4), five (n=5) and eight (n=3) stages of epiphyseal union (Figure 3.8.3.E). Two of the remaining methods were

based on the morphological features of skeletal elements, such as the sacrum (method 4) and sternum (method 28), and another two methods were based on morphometric traits of long bones (methods 7 and 13) (Figure 3.8.3.D).

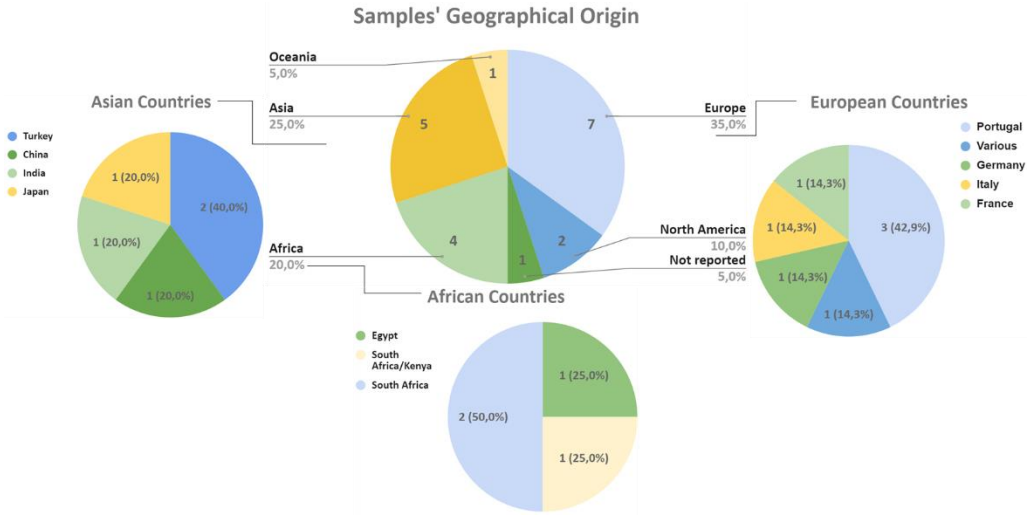

Figure 3.8.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_573. In the centre pie chart, the continent distribution is represented, followed by the pie charts of European and Asian countries (both countries with higher frequency in the centre pie chart).*

Group_575_Anatomical_areas: A total of 70% of these methods (n=14) used anatomical areas from the appendicular skeleton (Figure 3.8.4.): six methods (30%) used the upper limb (medial clavicle – n=4; humerus and/or scapula – n=2); six methods used the lower limb (iliac crest – n=4; femur and/or tibia and fibula – n=2). Four other methods used skeletal elements from the axial skeleton (Figure 3.8.4.): one method used the rib cage (more specifically, the sternum); three methods used the vertebral column (cervical vertebrae – n=1; sacrum – n=2). The two remaining methods were based on a combination of anatomical areas from the axial and appendicular skeleton (methods 1 and 5) (Figure 3.8.4.). A total of 30% of the methods (n=6) from Group_575 used a combination of skeletal elements, with the remaining fourteen methods focusing on only one.

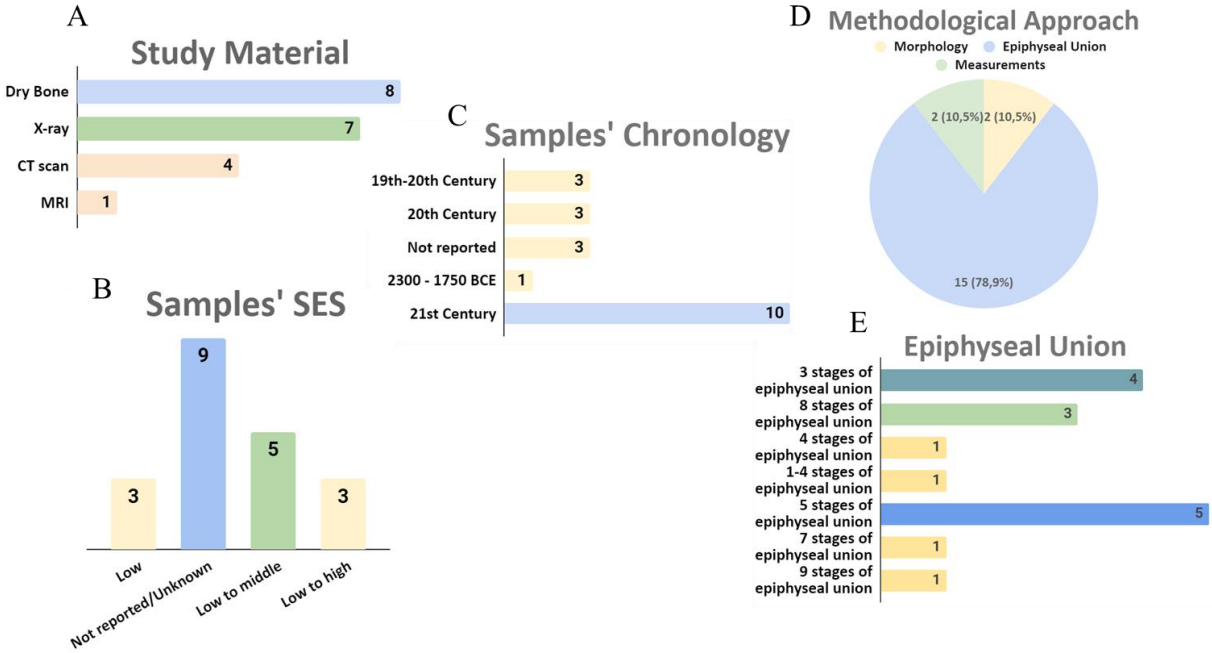

Figure 3.8.3.: *Charts regarding information about the BD methods from Group_575: A – bar chart of the study materials used; B – bar chart of the samples' SES; C – bar chart of the samples' chronology; D – pie chart of the methodological approach employed; E – bar chart regarding the stages of epiphyseal union used.*

Group_575_Accuracy: From the total of twenty BD methods from Group_575, only three reported values on accuracy (methods 4, 26 and 28). The other seventeen methods only provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.18).

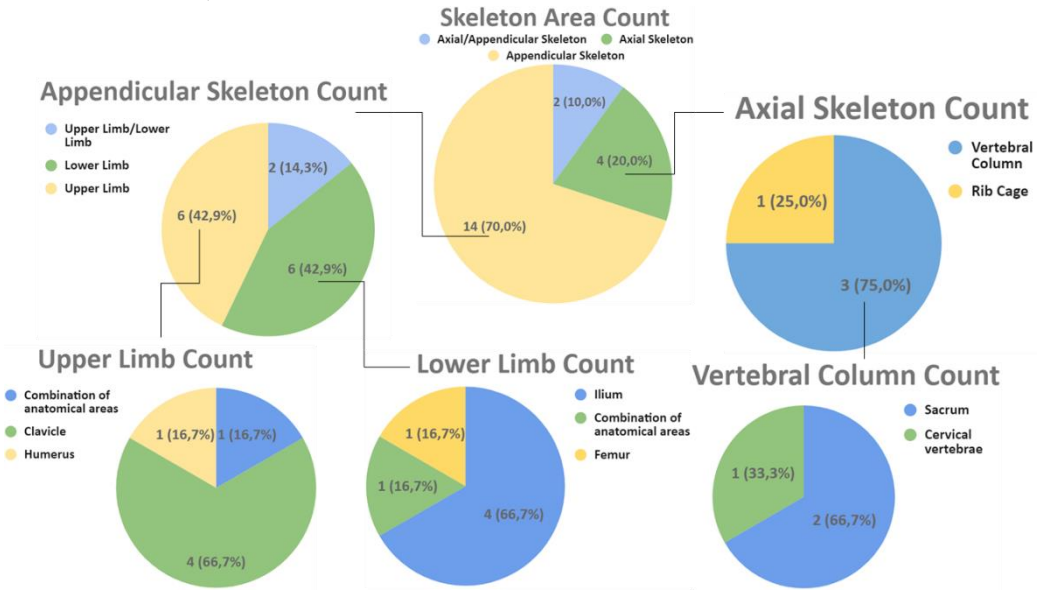

Figure 3.8.4.: *Pie charts representing the frequency of each skeleton area used in the BD methods from Group_575.*

575_SIC: Individual [575] presented three different types of SIC: LEH, CH and PR. LEH was present in both maxillary and mandibular dentition, and although it was more visible in the incisors and canines, every permanent tooth presented at least one LEH (including the third molars which were unerupted and the root was still open at time of death). A possible initial stage of CH was visible on the right humerus, with a diameter of around 0,5 cm. PR was observed in the form of woven and lamellar bone across the long bones from the upper and lower limb.

3.9. [872]

For individual [872], an AAD estimate of 0.5-2.5 years was obtained. Only a broad estimate could be obtained, since it was based only on method 7 (Figure 3.9.1.). This method was considered the reference for this individual, since it was the only BD method that gave a coincident AAD estimate to DD methods in individuals [575] and [1008] (the only other two individuals from the HRTS that present earlier stages of epiphyseal fusion). Biological sex was not estimated since the three bones from the *Os coxae* had not started fusing.

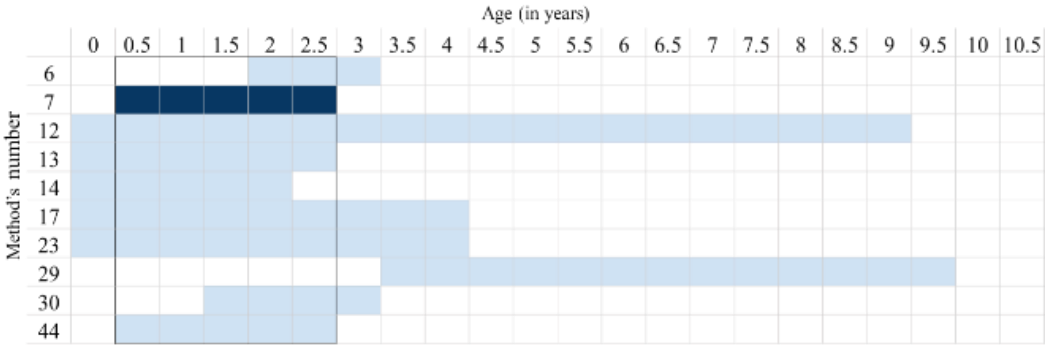

Figure 3.9.1.: *Diagram representing the AAD estimate provided by every method applied to individual [872]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the reference method is represented by the bar in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval obtained by the reference method AAD estimate.*

From the initial forty-four BD methods (the total fifty BD methods, minus the six BD methods used as reference in individuals without dentition – Chapter 3.3. for more details) only nine could be applied, due to the low preservation and conservation rates of this set of human skeletal remains and earlier stages of bone development. From these nine methods, eight gave an AAD estimate that coincided (either partially or completely) with the estimate given by the reference method, which corresponds to a total of 88.9% of the methods applied (Figure 3.9.1.). Hereon, the group composed by these eight methods will be referred to as **Group_872**.The only method that does not integrate Group_872 (method 29) overestimated AAD. Age ranges provided by the methods from Group_872 ranged between a 3-year (method 6) and a 10-year interval (method 12), with an average 4.25-year interval.

Group_872 includes methods 6, 12, 13, 14, 17, 23, 30 and 44 (see Appendix A, Table A.19 for a correspondence between methods codes and their authors and year of publication).

Group_872_Samples: All the samples used in these methods are European, with Portugal as the most common country of origin (n=3) (Figure 3.9.2.A). Samples chronology ranged between the 11th (n=1) and the 21st Century (n=1), with 37,5% (n=3) of the samples dated from the 20th Century (Figure 3.9.2.D). SES was described either as "Low" (n=1) or as "Low to middle" (n=4). In the remaining three methods, samples' SES was either not reported or not known (Figure 3.9.2.B).

Group_872_Study_materials: Six of these methods were developed based on dry bone samples, and the remaining two used either X-ray or CT scan samples (Figure 3.9.2.C).

Group_872_Methodological_approach: Five of the methods from Group_872 used metric traits of long bones to estimate AAD. The remaining three methods used either 3, 4 or 6 stages of epiphyseal union to estimate AAD (Figures 3.9.2.E and 3.9.2.F).

Group_872_Anatomical_areas: Half of the methods (n=4) from Group_872 used skeletal elements from the appendicular skeleton (Figure 3.9.3.): two methods used skeletal elements from the lower limb (ischium and pubis: n=1; ilium: n=1); two methods used a combination of skeletal elements from the upper and lower limb (methods 13 and 23). Two methods used anatomical areas from the axial skeleton (occipital bone; n=1; sacrum=1), and another two used a combination of skeletal elements from

the axial and appendicular skeleton (Figure 3.9.3.). From the total of eight BD methods from Group_872, five used a combination of skeletal elements, while the other three focused on one anatomical area to estimate AAD.

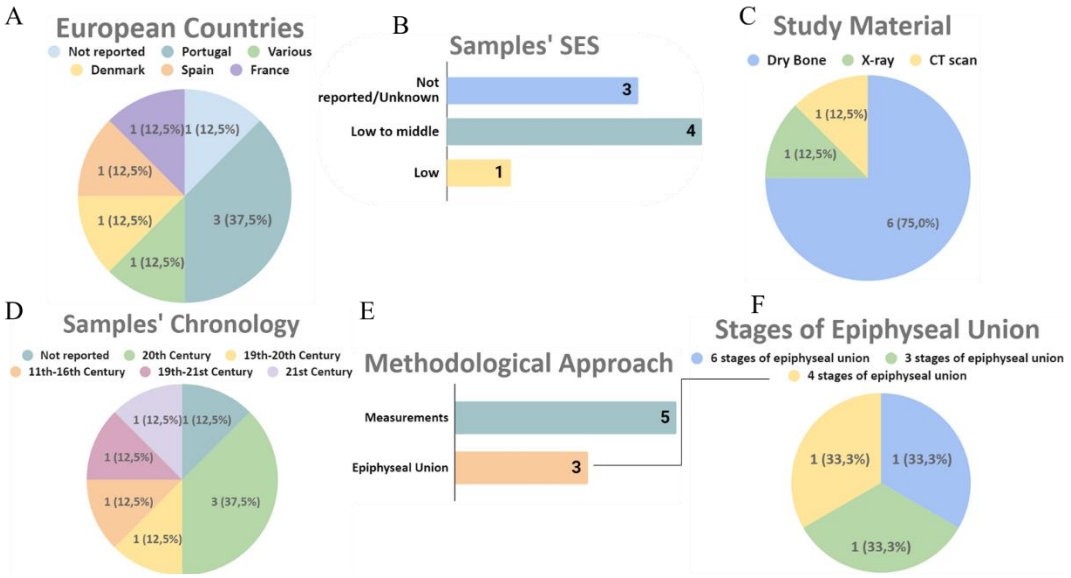

Figure 3.9.2.: *Charts regarding information about the BD methods from Group_872: A – pie chart of the samples' geographical origin (European countries); B – bar chart of the samples' SES; C – pie chart of the study materials used; D – pie chart of the samples' chronology; E – bar chart of the methodological approach employed; F – pie chart regarding the stages of epiphyseal union used.*

Group_872_Accuracy: Method's accuracy was only reported in method 44. The other seven methods only provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.19).

872_SIC: Individual [872] was one of the sets of human skeletal remains that presented the least number of SIC: only PR was registered for this individual. This may be a result of the low rates of preservation and conservation of this skeleton, which may translate into a real higher rate SIC that could not be observed. PR (mainly woven bone) was common across most long bones but was also observed in bones such as the vertebrae, scapula, ilium, ischium and pubis, with areas in which microporosity and/or woven bone was visible.

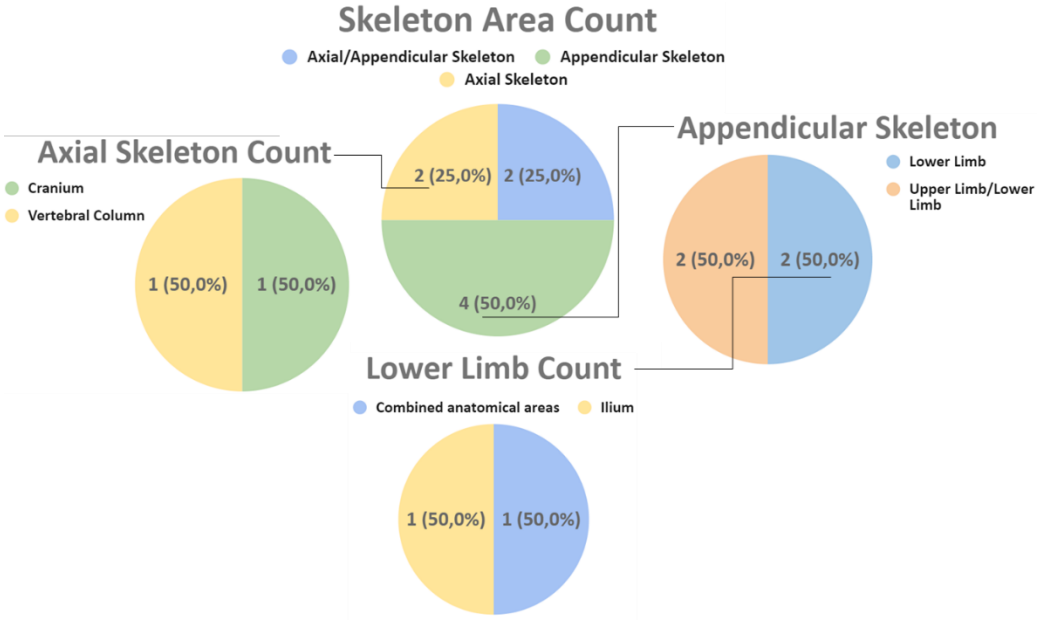

Figure 3.9.3.: *Pie charts representing the frequency of each skeleton area used in the BD methods from Group_872.*

3.10. [1008]

For individual [1008], an overall AAD estimate of 5.5-6 years was obtained based on the intersection of DD methods (Figure 3.10.1.). Biological sex was not estimated due to lack of fusion of the three bones from the *Os coxae*.

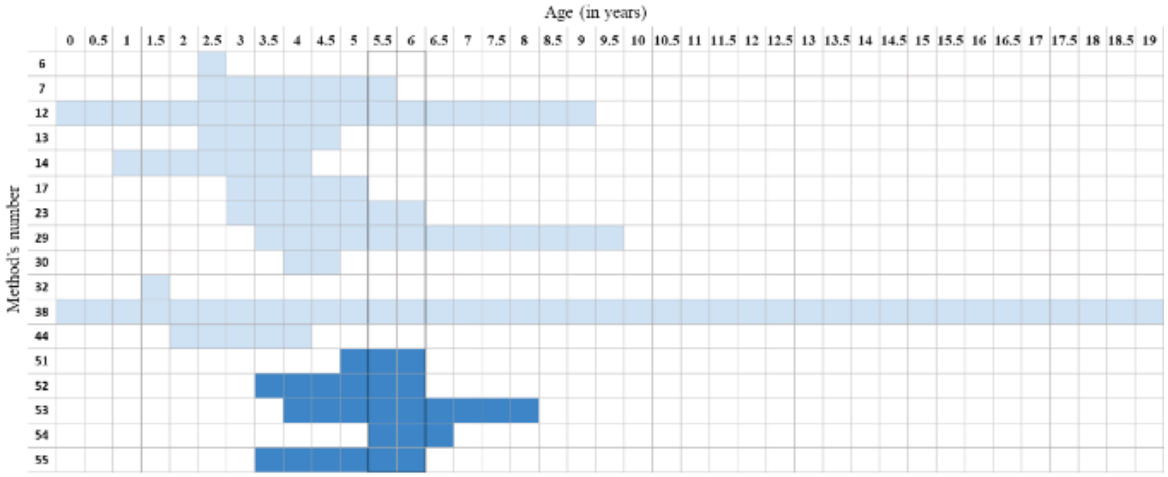

Figure 3.10.1.: *Diagram representing the AAD estimate provided by every method applied to individual [1008]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which every DD method overlap.*

From the initial fifty BD methods selected, a total of twelve could be applied to this individual, due to general BD and to low conservation and preservation rates. From these twelve methods, only five gave an AAD estimate that coincided (either partially or completely) with the estimate given by the DD methods, corresponding to a total of 41.7% of the methods applied (Figure 3.10.1). Hereon, the group composed by these five BD methods will be referred to as **Group_1008**. All the other seven BD methods tended to underestimate AAD. Group_1008 methods' provided age ranges that varied between a 4-year (method 7 and 23) and a 20-year interval (method 38), with an average of a 9-year interval.

Group_1008 includes methods 7, 12, 23, 29 and 38 (see Appendix A, Table A.20 for a correspondence between methods codes and their authors and year of publication).

Group_1008_Samples: Three of these methods' samples (60%) are European (Portugal: n=2; Denmark: n=1), while the remaining two are African (Egypt) and from Oceania (Australia) (Figure 3.10.2.). Samples chronology changed between 2300-1750 BCE (n=1) and the 21st Century (n=1), with 40% (n=2) dated from the 20th Century (Figure 3.10.3.C). SES was described either as "Low" (n=1) or as "Low to middle" (n=3). SES was only reported as unknown or not reported in one method (Figure 3.10.3.D).

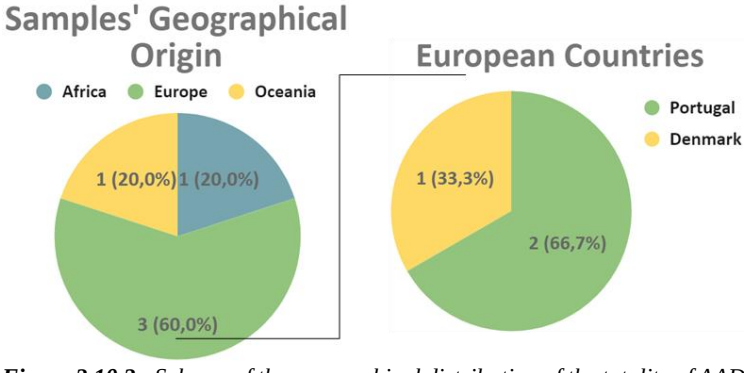

Figure 3.10.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_1008. In the left pie chart, the continent distribution is represented, followed by the pie chart of European countries.*

Group_1008_Study_material: The most commonly used study material in Group_1008 is dry bone samples (n=3), followed by X-ray (n=1) and CT scan (n=1) samples (Figure 3.10.3.E).

Group_1008_Methodological_approach: A total of 40% (n=2) of these methods used metric traits of the long bones to estimate AAD, while the remaining 60% (n=3) used different stages of epiphyseal union: the 3 stages (n=2) and the 6 stages (n=1) of epiphyseal union (Figure 3.10.3.B).

Group_1008_Anatomical areas: A total of 80% (n=4) of these methods used anatomical areas from the appendicular skeleton (Figure 3.10.3.A): two methods were based on the lower limb (ischiopubic ramus: n=1; *Os coxae* and femur: n=1); another two methods used a combination of skeletal elements from the upper and lower limb (methods 7 and 23). Only one method was based on the axial skeleton (sternum: n=1). This was also the only BD method using a single skeletal element, the other four methods used a combination of different anatomical areas (Figure 3.10.3.A).

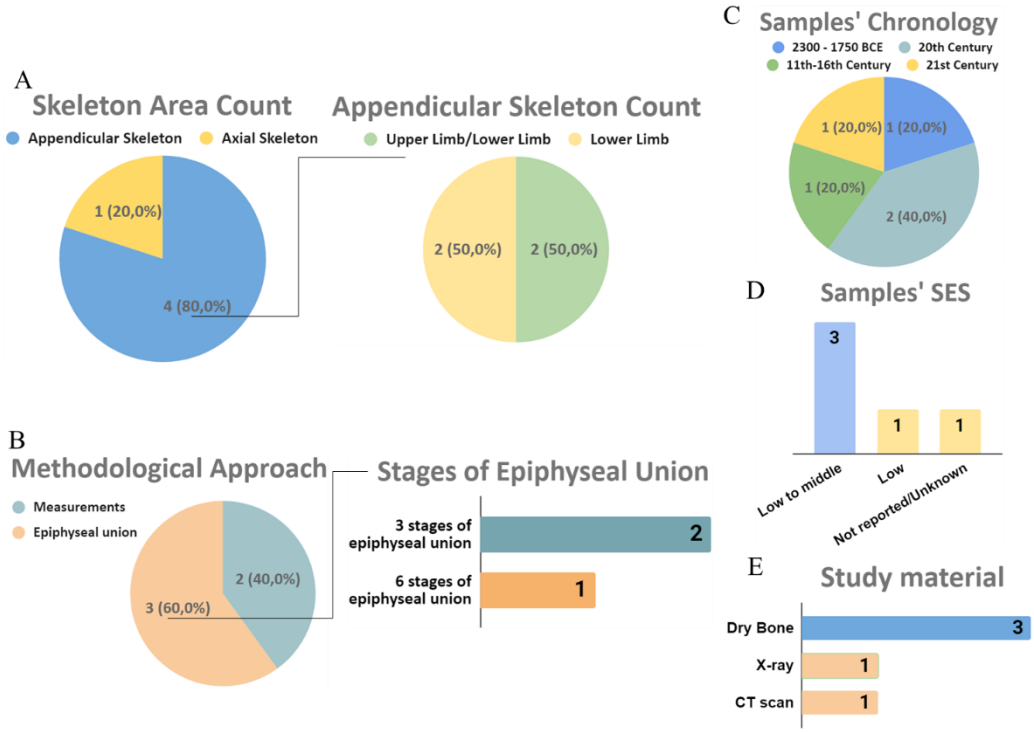

Figure 3.10.3.: *Charts regarding information about the BD methods from Group_1008: A - Pie charts representing the frequency of each skeleton area used in the BD methods; B - pie chart of the methodological approach employed and bar chart of the stages of epiphyseal union; C – pie chart of the samples' chronology; D – bar graph of the samples' SES; E – bar graph of the study materials used.*

Group_1008_Accuracy: None of the five methods from Group_108 provided values for accuracy. Only values for intra- and inter-observer agreement and other parameters were presented (for more detailed information and values, see Appendix A, Table A.20).

1008_SIC: Individual [1008] presented three types of SIC: PR, LEH and CF. LEH was present in mandibular dentition, both in decidual and definitive teeth (which were unerupted and presented an open root), being more expressive in the latter. CF could only be observed in the right femur since the left femur was not recovered. PR, consisting mainly of microporosity and/or woven bone, was observed in the diaphysis of most long bones from the upper and lower limb, but was also observed in the vertebrae bodies and the pubis, ilium, and ischium.

3.11. [1214]

An overall AAD estimate of >21 years was obtained for individual [1214], based on DD methods' intersection (Figure 3.11.1.). Biological sex was estimated as male, based on the morphology of the os coxae and skull.

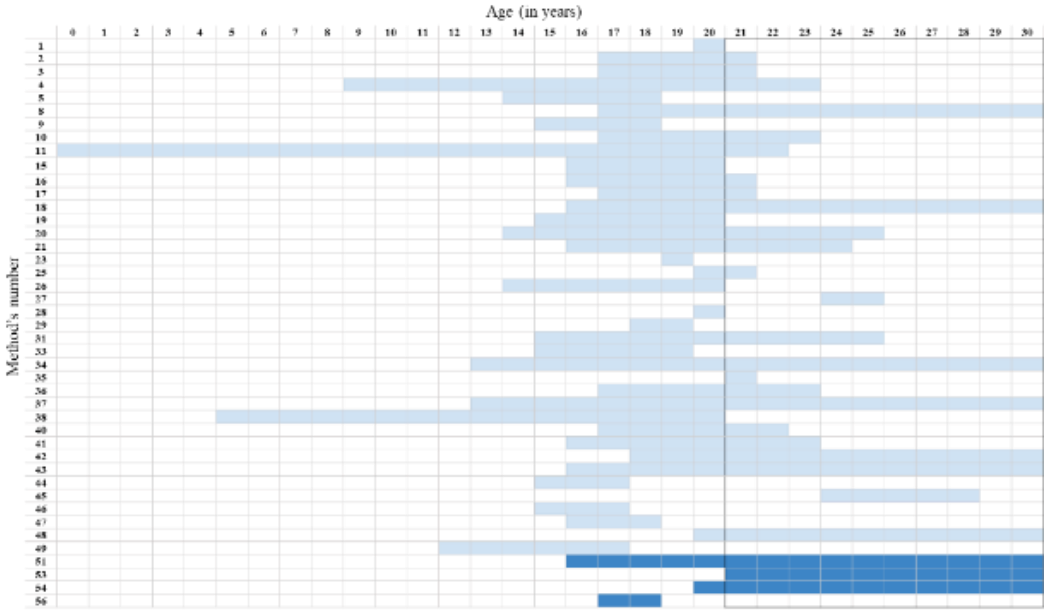

Figure 3.11.1.: *Diagram representing the AAD estimate provided by every method applied to individual [1214]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which every DD method overlap.*

From the initial fifty BD method, a total of forty-three methods could be applied, either due to the overall stages of BD and/or rates of preservation and conservation of the skeleton. From these forty-three methods, only twenty-four gave a similar AAD estimate to the DD methods, corresponding to a total of 55.8% of the methods applied. Hereon, the group composed by these twenty-four BD methods will be referred to as **Group_1214**. All of the remaining BD methods underestimated AAD of this set of human skeletal remains. Age ranges obtained from the Group_1214's methods varied between a 1-year (method 35) and a 23-year interval (method 11), with an average of a 9.71-year interval (Figure 3.11.1.).

Group_1214 includes methods 2, 3, 4, 8, 10, 11, 16, 17, 18, 20, 21, 25, 27, 31, 34, 35, 36, 37, 40, 41, 42, 43, 45 and 48 (see Appendix A, Table A.21 for a correspondence between methods codes and their authors and year of publication).

Group_1214_Samples: A total of 41,7% (n=10) of these methods samples are European, with Portugal being the most frequent country of origin

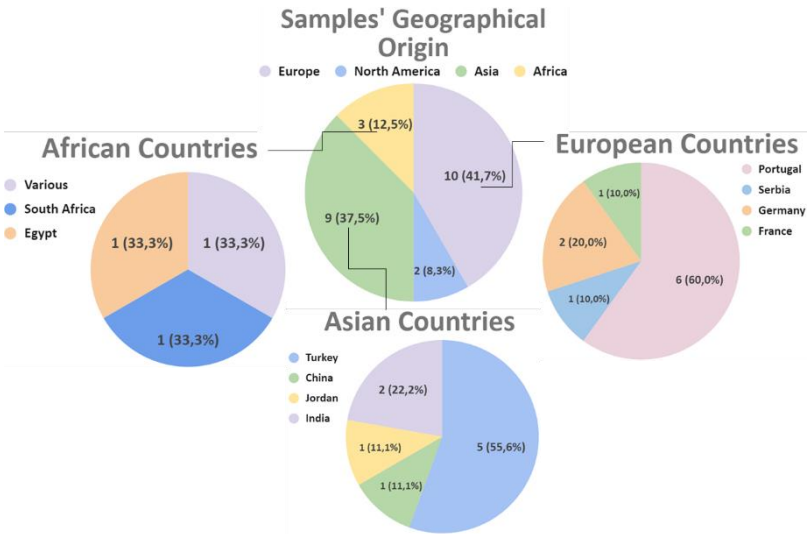

Figure 3.11.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_573. In the centre pie chart, the continent distribution is represented, followed by the pie charts of European, African, and Asian countries distribution.*

(n=6). This was followed by Asian countries (n=9) – in which the most common origin was Turkey (n=5) – and African countries (n=3). Only two methods used North American (USA) samples (Figure 3.11.2.). Samples' chronology ranged between the 19th (n=1) and the 21st Century, with 54.2% (n=13) dated from the latter (Figure 3.11.3.D). SES was described either as "Low" (n=3), as "Low to middle" (n=7), as "Low to high" (n=1), "Middle" (n=1) or as "Middle to high" (n=1). The remaining eleven methods either did not report or did not have access to each samples' SES (Figure 3.11.3.B).

Group_1214_Study_materials: The most common study material was dry bone (n=8), followed by X-ray (n=5), MRI (n=5) , CT scan (n=4) and human cadavers (n=2) samples (Figure 3.11.3.A).

Group_1214_Methodological_approach: A total of 87.5% (n=21) of these methods used different stages of epiphyseal union, in which the more common were the 3 (n=6), 5 (n=8), and 8 (n=4) stages of epiphyseal union (Figure 3.11.3.E). The remaining three methods were based on the morphology of different skeletal elements, such as the sacrum (method 4), the medial clavicle (method 22) and the combination of the spheno-occipital synchondrosis, the third molar and the cervical vertebrae (method 27) (Figure 3.11.3.C).

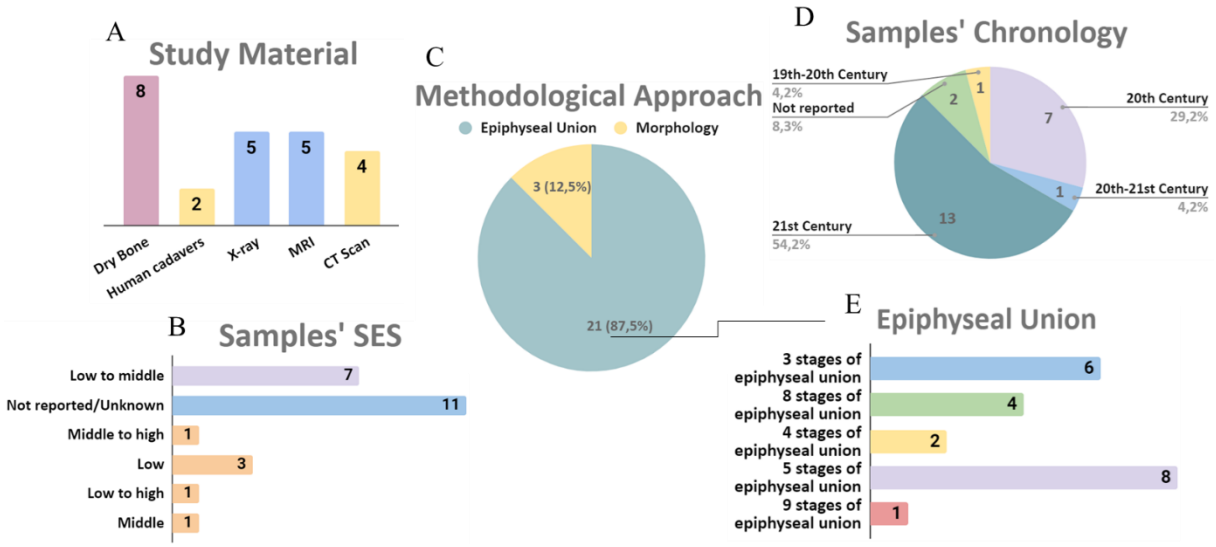

Figure 3.11.3.: *Charts regarding information about the BD methods from Group_1214: A – bar chart of the study materials used; B – bar chat of the samples' SES; C – pie charts of the methodological approaches employed; D – pie chart of the samples' chronology; E – bar chart of the stages of epiphyseal union used.*

Group_1214_Anatomical_areas: a total of 75% (n=18) of these methods were based on skeletal elements from the appendicular skeleton (Figure 3.11.4.): nine methods were based on the upper limb (medial: n=4; scapula: n=1; proximal humerus: n=2; combination of skeletal elements: n=2); eight methods were based on the lower limb (iliac crest: n=3; tibia: n=2; femur: n=1; combination of skeletal elements: n=2); one method used a combination of anatomical areas from the upper and lower limb (method 8). The other six methods were based on the axial skeleton (Figure 3.11.4.): two methods were based on the cranium (spheno-occipital synchondrosis – methods 34 and 35); three methods were based on the vertebral column (sacrum: n=2; presacral vertebra: n=1); one method was based on the combination of the cranium, dentition, and vertebral column (method 27). No method used a combination of skeletal elements from the appendicular and axial skeleton (Figure 3.11.4.). Furthermore, a total of 75% (n=18) of the methods from Group_1214 focused on only one skeletal element to provide an AAD estimate, while only the remaining 25% (n=6) opted for a combination of different anatomical regions.

Group_1214_Accuracy: From the total twenty-four methods from Group_1214, only one reported values on accuracy (method 4). The other twenty-three methods provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.21).

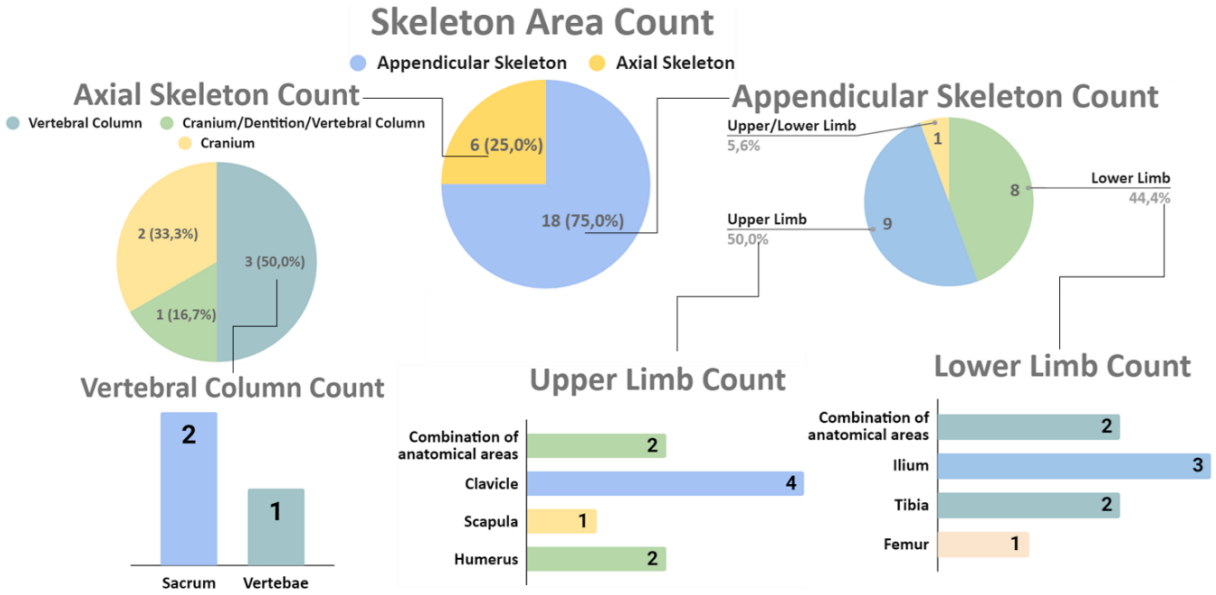

Figure 3.11.4.: *Pie and bar charts representing the frequency of each skeleton area used in the BD methods from Group_1214.*

1214_SIC: Individual [1214] was the one with the higher number of SIC observed: LEH, PR, CF and CH. CF was observed bilaterally, although it could not be observed to its full extent in the right femur due to its preservation state. CH was more visible in the right humerus, although possibly at its healing state. LEH was observed both in mandibular and maxillary dentition, with more than one line visible in the incisors and canines and at least one in each molar. PR was observed in the form of lamellar bone across the diaphysis of the long bones from the upper and lower limb, although its incidence was higher in the bones from the lower limbs (especially the femur and tibia).

3.12. [1296]

For individual [1296], an overall AAD estimate of >21 years was obtained, based on DD methods' intersection (DD was complete) (Figure 3.12.1.). Biological sex was estimated as female, based on the morphology of the os coxae and skull.

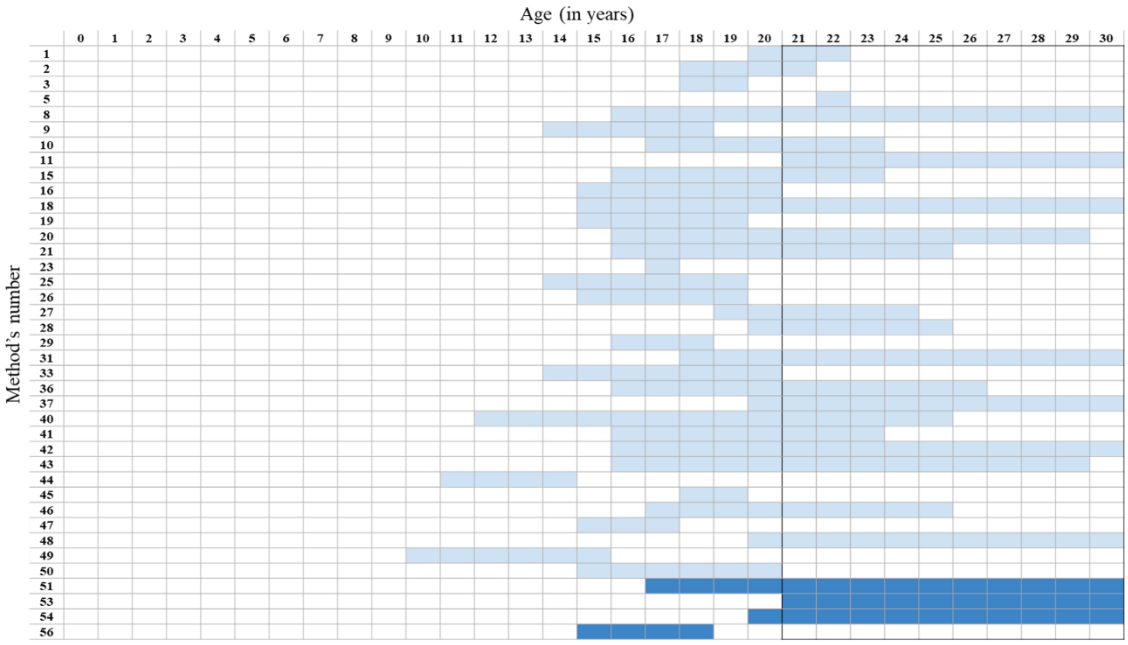

Figure 3.12.1.: *Diagram representing the AAD estimate provided by every method applied to individual [1296]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which every DD method overlap.*

From the initial fifty BD methods, only thirty-five could be applied to this set of human skeletal remains, either due to the overall stages of BD and/or rates of preservation and conservation of the skeleton. From these thirty-five methods, twenty-one gave a coincident AAD estimate (either partially or completely) to the DD methods, corresponding to a total of 60% of the methods applied. Hereon, the group composed by these twenty-one BD methods will be referred to as **Group_1296**. All the remaining methods underestimated AAD when compared to DD methods. Age ranges provided by the methods from Group_1296 varied between a 1-year (method 5) and a 16-year interval (method 18), with an average of a 9.81-year interval (Figure 3.12.1.).

Group_1296 includes methods 1, 2, 5, 8, 10, 11, 15, 18, 20, 21, 27, 28, 31, 36, 37, 40, 41, 42, 43, 46 and 48 (see Appendix A, Table A.22 for a

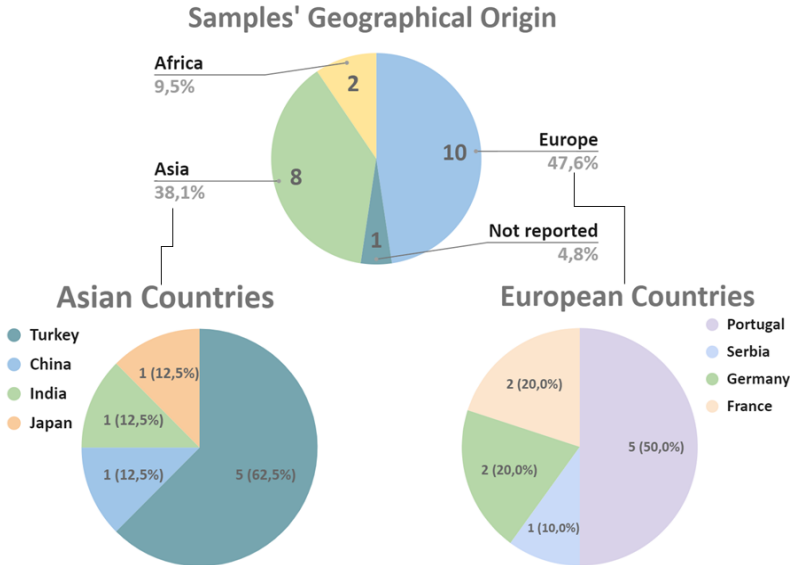

Figure 3.12.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_1296. In the centre pie chart, the continent distribution is represented, followed by the pie charts of European and Asian countries.*

correspondence between methods codes and their authors and year of publication).

Group_1296_Samples: A total of 47.6% (n=10) of the samples were European (half of these from Portugal), and 38.1% (n=8) were Asian (more than half from Turkey: n=5). The remaining three samples were African (Kenya and/or South Africa; n=2) and American (USA; n=1) (Figure 3.12.2.). Samples' chronology ranged between the 19th (n=2) and the 21st Century, with more than half (n=12) of the samples dated from the current century (Figure 3.12.3.B). SES was either described as "Low" (n=4), as "Low o middle" (n=5), as "Middle to high" (n=2) or as "Low to high" (n=1). The remaining nine methods either did not report or did not know the samples' SES (Figure 3.12.3.C).

Group_1296_Study_materials: The study materials most frequently used were dry bone samples (n=7), followed by MRI (n=5), CT scan (n=5), X-rays (n=3) and human cadaver (n=1) samples (Figure 3.12.3A).

Group_1296_Methodological_approach: A total of 85.7% (n=18) of the methods from Group_1296 used different stages of epiphyseal union to estimate AAD, from which the most common were the 3 (n=6), 5 (n=7) and 8 (n=3) stages of epiphyseal union (Figure 3.12.3.E). The remaining three methods were based on the morphology of different skeletal elements, such as the medial clavicle (method 11), the sternum (method 28) and a combination of the spheno-occipital synchondrosis, the third molar and the cervical vertebrae (method 27) (Figure 3.12.3.D).

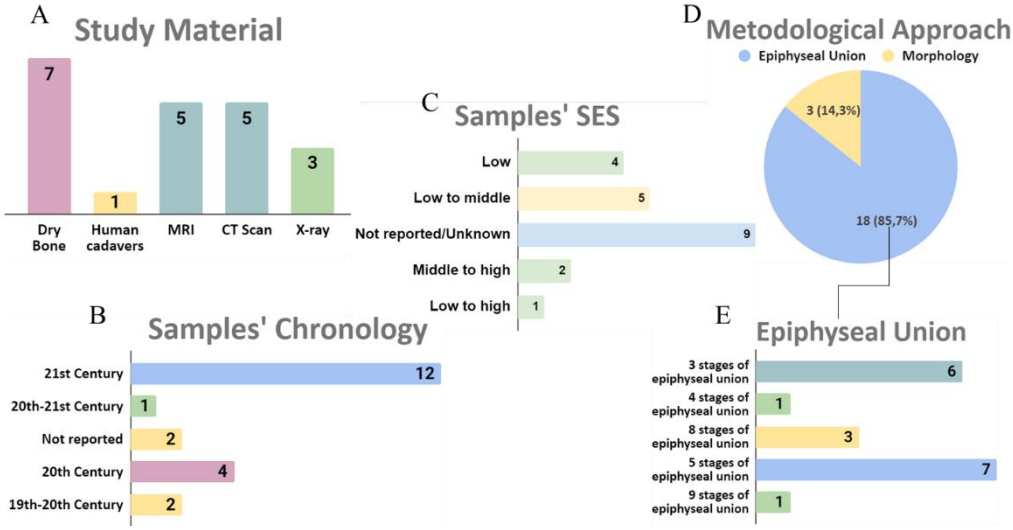

Figure 3.12.3.: *Charts regarding information about the BD methods from Group_1296: A – bar chart of the study materials used; B – bar chart of the samples' chronology; C – bar chart of the samples' SES; D – pie chart of the methodological approach employed; E – bar chart regarding the stages of epiphyseal union used.*

Group_1296_Anatomical_areas: 76.2% (n=16) of the methods from Group_1296 used anatomical regions from the appendicular skeleton (Figure 3.12.4.): nine methods were based on the upper limb (medial clavicle: n=6; proximal humerus: n=2; scapula: n=1); six methods were based on the lower limb (iliac crest: n=2; distal femur: n=1; proximal tibia: n=1; combination of skeletal elements: n=2); one method used a combination of the upper and lower limb (method 8). Three methods were based on the axial skeleton (presacral vertebrae: n=1; sternum: n=1; spheno-occipital synchondrosis, third molar and cervical vertebrae: n=1), and another two were based on a combination of skeletal elements from the axial and appendicular skeleton (methods 1 and 5) (Figure 3.12.4.). A total of 71.43% (n=15) of the methods from Group_1296 focused on a single skeletal element to estimate AAD, while the remaining used two or more anatomical areas.

Group_1296_Accuracy: From the total of twenty-one methods from this group, only one BD method (method 28) reported values on accuracy. The other twenty methods only provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.22).

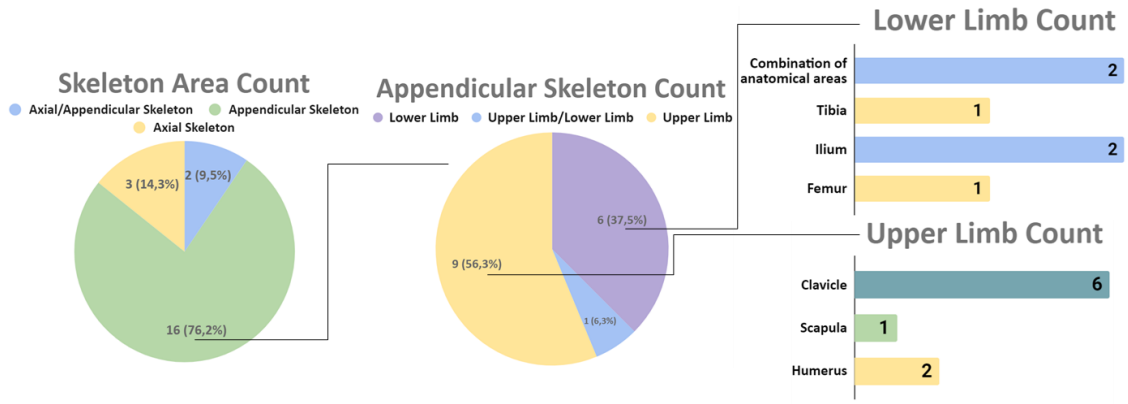

Figure 3.12.4.: *Pie charts and bar charts representing the frequency of each skeleton area used in the BD methods from Group_1296.*

Group_1296_SIC: Three different types of SIC were observed in individual [1296]: LEH, CF, and PR. LEH was observed in both mandibular and maxillary dentition, with higher incidence on the incisors and canines (at least one line visible in each of these). LEH were also present in premolars and molars, although to a lesser extent. CF was observed bilaterally, even though its appearance was much more discrete than in some of the other individuals (for example, [573, [575] or [1008]). PR was mainly present in the long bones and was more visible in the upper limb. It was mainly observed under the form of a healed lesion, with some lamellar bone still visible in some areas.

3.13. [1313]

For individual [1313], an overall AAD estimate of 16-25 years was obtained based on the reference BD methods (as previously explained, these methods correspond to Group_Dentition1 and were used as the reference methods in individuals without dentition preserved) (Figure 3.13.1). Biological sex was indeterminate due to the low rates of preservation and conservation of this set of human skeletal remains.

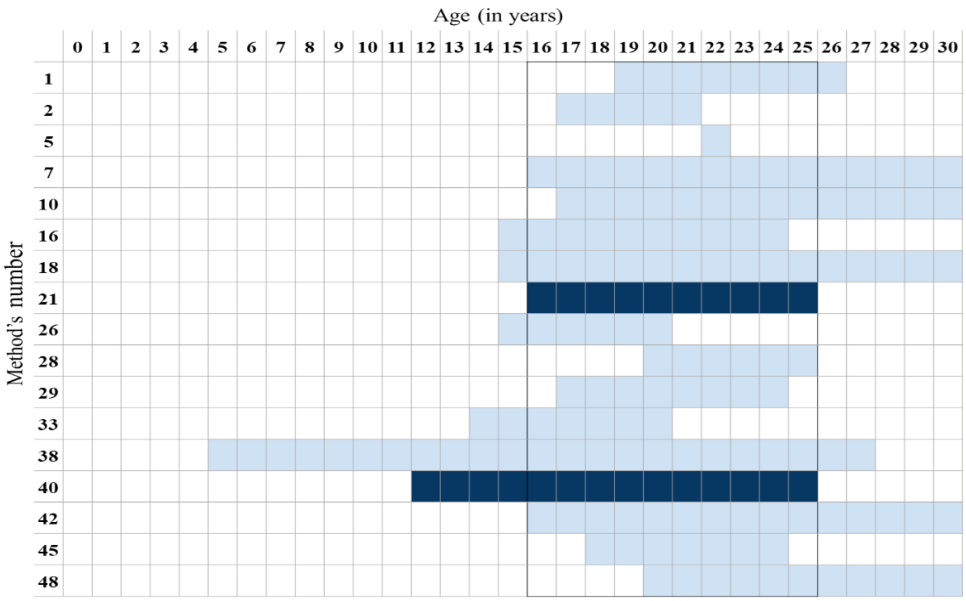

Figure 3.13.1.: *Diagram representing the AAD estimate provided by every method applied to individual [1313]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the reference BD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which the reference BD methods overlap.*

From the initial forty-four BD methods (the total fifty BD methods, minus the six BD methods used as reference in individuals without dentition – Chapter 3.3. for more details), only fifteen could be applied either due to the overall stages of BD and/or rates of preservation and conservation of the skeleton. The totality of the fifteen BD methods gave a coincident (either partially or completely) AAD estimate to the reference BD methods. Hereon, the group composed by these fifteen BD methods will be referred to as **Group_1313**. Age ranges provided by these methods varied between a 1-year (method 5) and a 23-year interval (method 38), with an average 10.13-year interval (Figure 3.13.1.).

Group_1313 includes methods 1, 2, 5, 7, 10, 16, 18, 26, 28, 29, 33, 38, 42, 45 and 48 (see Appendix A, Table A.23 for a correspondence between methods codes and their authors and year of publication).

Group_1313_Samples: 60% of these methods' samples (n=9) are European, with Portugal as the most common country of origin (n=5). This was followed by Africa (n=2), Oceania (n=2), and Asia (n=1) (Figure 3.13.2). Samples' chronology ranged between 2300-1750 BCE (n=1) and the 21st Century, with the latter being the most common (n=8) (Figure 3.13.3.C). SES was either described as "Low" (n=1) or as "Low to middle" (n=4). The remaining ten methods either did not report or did not have access to the samples' SES (Figure 3.13.3.B).

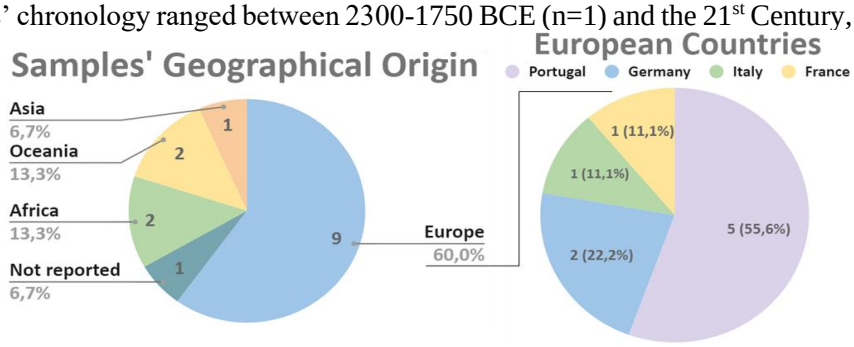

Figure 3.13.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_1313. In the left pie chart, the continent distribution is represented, followed by the pie chart of European countries on the right.*

Group_1313_Study_materials: The most frequently used study materials were dry bone samples (n=7), followed by MRI (n=3), CT scan (n=3) and X-ray (n=2) (Figure 3.13.3.A).

Group_1313_Methodological_approach: A total of 86.7% (n=13) of the methods from Group_1313 used different stages of epiphyseal union to estimate AAD, from which the 3 (n=7), 5 (n=2) and 8 (n=2) stages of epiphyseal union were the most common (Figure 3.13.3.E). From the two remaining methods, one was based on measures of the long bones (method 7) and the other was based on the morphology of the sternum (method 28) (Figure 3.13.3.D).

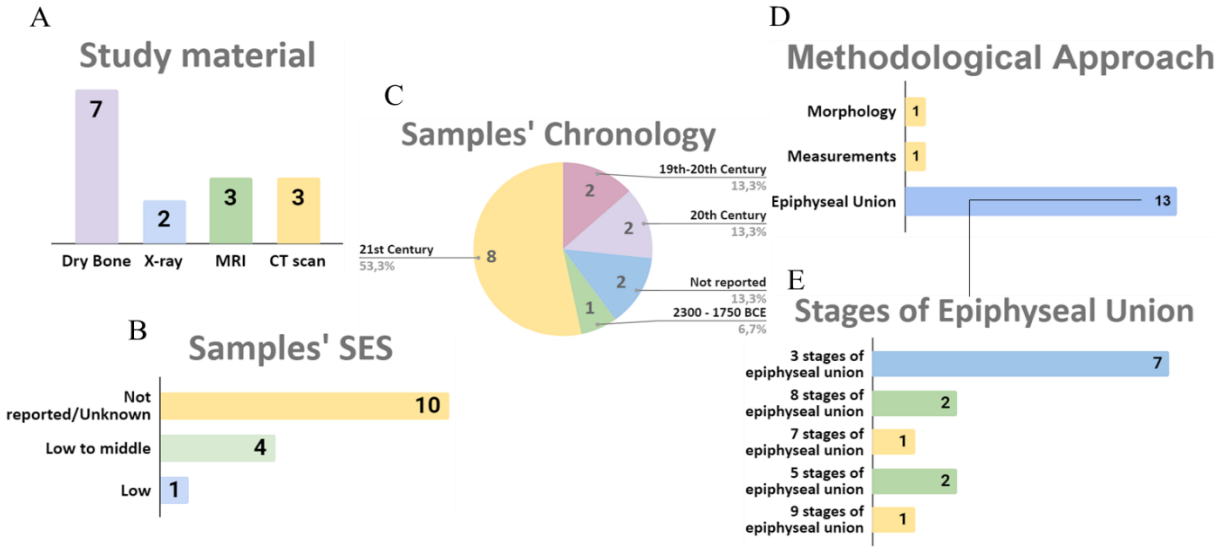

Figure 3.13.3.: *Charts regarding information about the BD methods from Group_1313: A – bar chart of the study materials used; B – bar chart of the samples' SES; C – pie chart of the samples' chronology; D – bar chart of the methodological approach employed; E – bar chart regarding the stages of epiphyseal union used.*

Group_1313_Anatomical areas: 66.7% (n=10) of these methods used anatomical areas from the appendicular skeleton (Figure 3.13.4.): nine methods were based on skeletal elements of the lower limb (iliac crest: n=2; proximal tibia: n=2; distal femur: n=1; combination of skeletal elements: n=4); one method used a combination of anatomical areas from the upper and lower limb (method 7). From the remaining five methods, three used anatomical areas from the axial skeleton (vertebrae: n=1; sternum: n=2) and two used a combination of skeletal elements from the axial and the appendicular skeleton (methods 1 and 5) (Figure 3.13.4.). 46.67% (n=7) of these methods used a combination of different skeletal elements to estimate AAD, while the remaining 53.33% (n=8) focused on only one to provide an estimate.

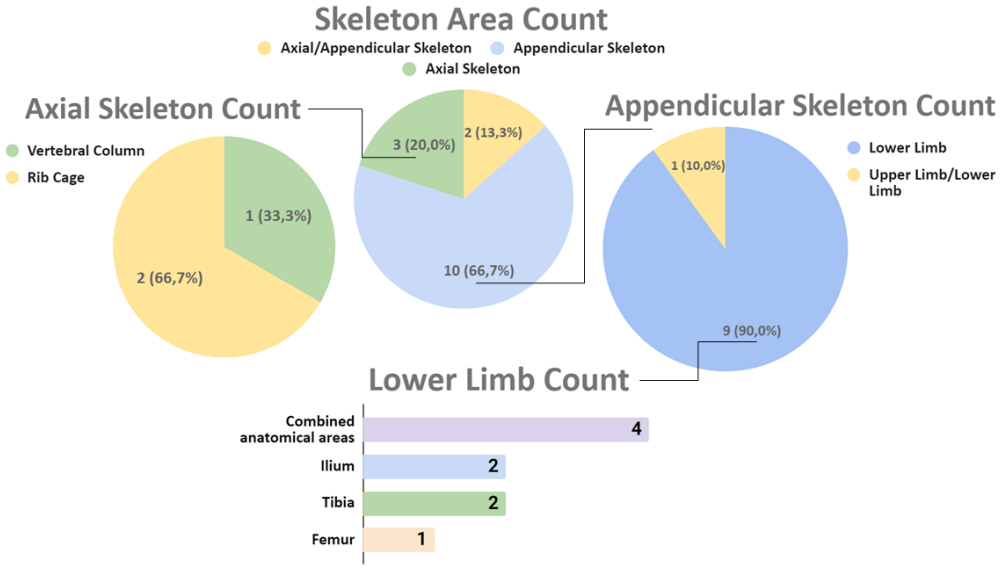

Figure 3.13.4.: *Pie charts representing the frequency of each skeleton area used in the BD methods from Group_1313.*

Group_1313_Accuracy: From the total of fifteen BD methods that compose Group_1313, only two (methods 26 and 28) reported values on accuracy. The other thirteen methods only provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.23).

1313_SIC: Only one form of SIC was observed in individual [1313]: PR. This is probably due to its preservation and conservation rates, since most of the other anatomical structures in which SIC were usually observed (cranium, dentition, and femora' and humerus' necks) were not present during analysis. PR was present across long bones' diaphysis, through the form of both woven and lamellar bone. In some areas, bone growth was considerable, expanding noticeably across the bones limits and altering its original shape (this was particularly the case in the distal fibulas' diaphysis).

3.14. [1314]

For individual [1314], an overall AAD estimate of 24-27 years was obtained based on DD methods' intersection (Figure 3.14.1.). Biological sex was estimated as male, based on skull's morphology and long bones measurements.

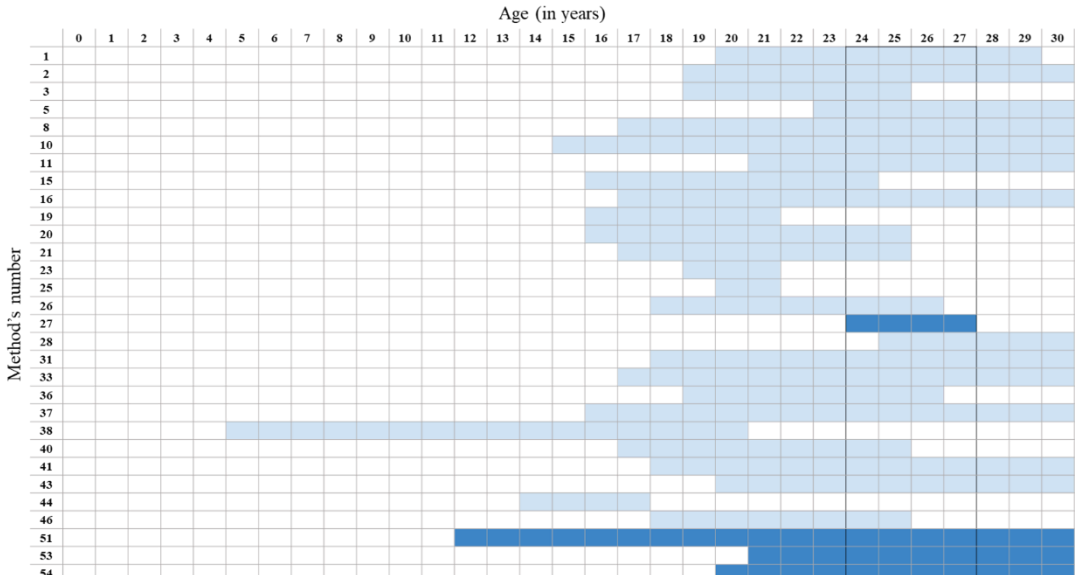

Figure 3.14.1.: *Diagram representing the AAD estimate provided by every method applied to individual [1314]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which every DD method overlap.*

From the initial fifty BD methods, only a total of twenty-six could be applied to this set of human skeletal remains, either due to the overall stages of BD and/or rates of preservation and conservation of the skeleton. From these twenty-six methods, twenty-one gave an AAD estimate that coincided (either partially or completely) with the estimate given by the DD methods, corresponding to a total of 80.77% of the methods applied. Hereon, the group composed by these twenty-one BD methods will be referred to as **Group_1314**. Age ranges provided by these methods varied between a 6-year (method 28) and a 16-year interval (method 10), with an average of a 10.71-year interval (Figure 3.14.1.).

Group_1314 includes methods 1, 2, 3, 5, 8, 10, 11, 15, 16, 20, 21, 26, 28, 31, 33, 36, 37, 40, 41, 43 and 46 (see Appendix A, Table A.24 for a correspondence between methods codes and their authors and year of publication).

Group_1314_Samples: 52.4% (n=11) of these methods' samples are European, with Portugal as the most common country (n=5). This is followed by Asian samples (n=6), with half of these originating from Turkey (n=3), and by African samples (n=2) (Figure 3.14.2.). Samples chronology ranged between the 19th (n=1) and the 21st

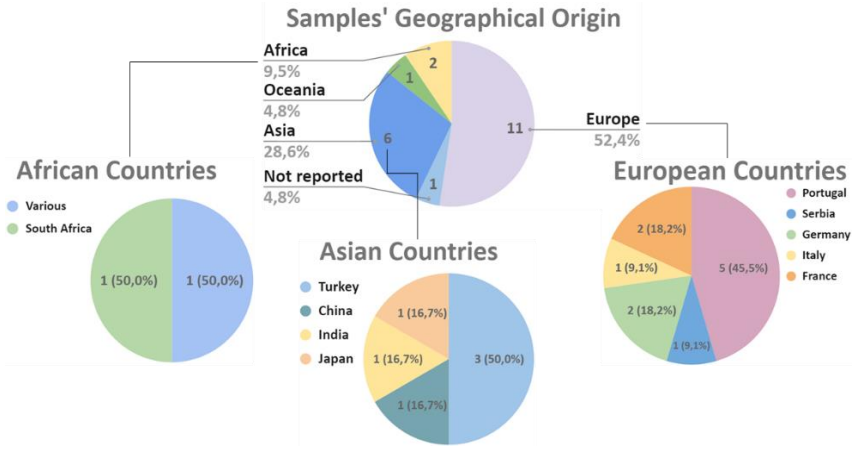

Figure 3.14.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_1314. In the centre pie chart, the continent distribution is represented, followed by the pie charts of European, African, and Asian countries*

Century, with more than half of these dated from the current century (n=12) (Figure 3.14.3.B). SES was described either as "Low" (n=4), as "Low to middle" (n=5), as "Middle to high" (n=2) or as "Low to high" (n=1). The remaining nine methods either did not report or did not know the samples' SES (Figure 3.14.3.A).

Group_1314_Study_materials: The most frequently used study materials were dry bone samples (n=7). This was followed by X-ray (n=5), CT scan (n=5), MRI (n=3), and human cadavers (n=1) samples (Figure 3.14.3.D).

Group_1314_Methodological_approach: A total of 90.5% (n=19) of the methods were based on different stages of epiphyseal union, in which the more common were the 3 (n=8), 5 (n=5) and 8 (n=3) stages of epiphyseal union (figure 3.14.3.E). The remaining two methods were based on the morphology of different skeletal elements, such as the medial clavicle (method 11) and the sternum (method 28) (Figure 3.14.3.C).

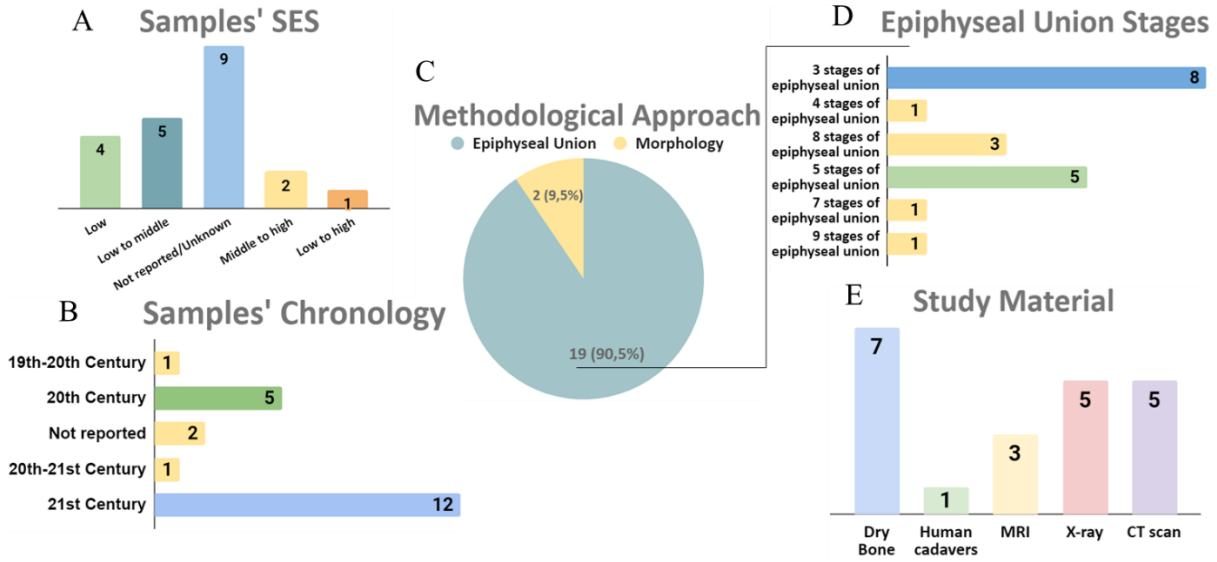

Figure 3.14.3.: *Charts regarding information about the BD methods from Group_1314: A – bar chart of the samples' SES; B – bar chart of the samples' chronology; C – pie chart of the methodological approaches employed; D – bar chart of the stages of epiphyseal union used; E – bar graph of the study materials used.*

Group_1314_Anatomical_areas: 81% (n=17) of the methods from Group_1314 used anatomical regions from the appendicular skeleton (Figure 3.14.4.): ten methods were based on the upper limb (medial clavicle: n=6; proximal humerus: n=2; scapula: n=1; combination of skeletal elements: n=1); six methods were based on the lower limb (iliac crest: n=4, combination of skeletal elements: n=2); one method was based on a combination of skeletal elements from the upper and the lower limb (method 8). Only two methods were based on the axial skeleton (presacral vertebrae: n=1; sternum: n=1), and the other two methods were based on a combination of anatomical areas from the axial and appendicular skeleton (methods 1 and 5) (Figure 3.14.4.). 71.4% (n=15) focused on only one skeletal element to estimate AAD, while the remaining 28.57% (n=6) of the methods used a combination of different anatomical regions.

Group_1314_Accuracy: From the total of twenty-one methods from Group_1314, only two methods (methods 26 and 28) reported values on accuracy. The other nineteen methods only provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.24).

1314_SIC: Two different types of SIC were identified in individual [1314] during analysis: LEH and PR. LEH was present in both mandibular and maxillary dentition, with higher incidence in incisors and canines (at least two LEH could be observed). However, these lesions were also visible in the premolars and molars to a lesser extent (at least one LEH). PR was mainly present in the long bones

from the upper and lower limb, visible as woven and lamellar bone, but also in bones such as the scapula and os coxae.

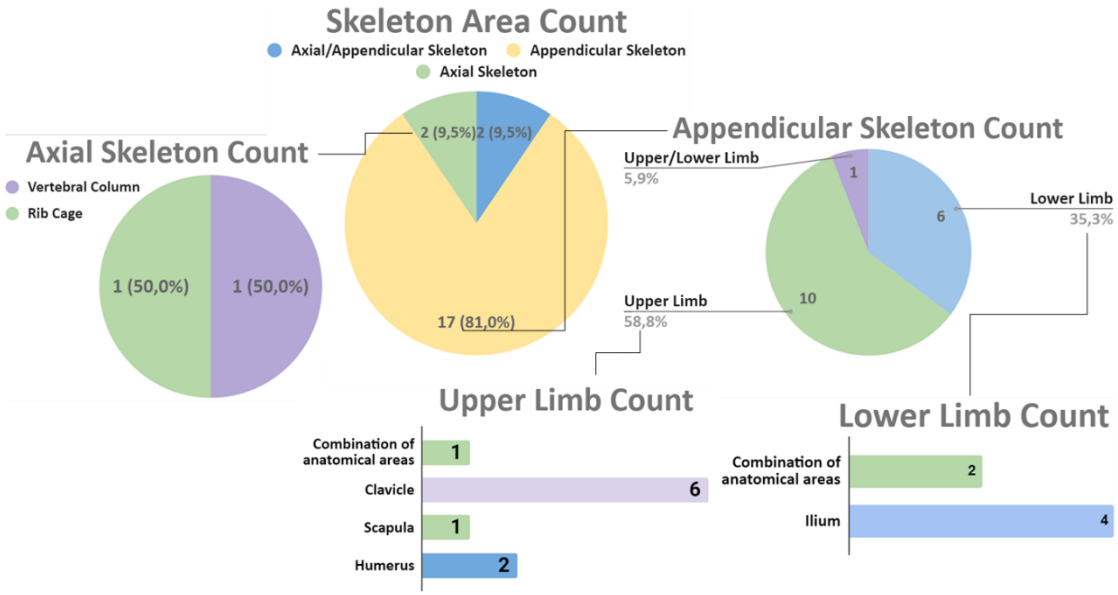

Figure 3.14.4.: *Pie charts representing the frequency of each skeleton area used in the BD methods from Group_1314.*

3.15. [1406]

For individual [1406], an overall AAD estimate of >21 years was obtained based on DD methods' intersection (Figure 3.15.1.). Biological sex for this individual was estimated as female, based on the morphology of the os coxae and the skull.

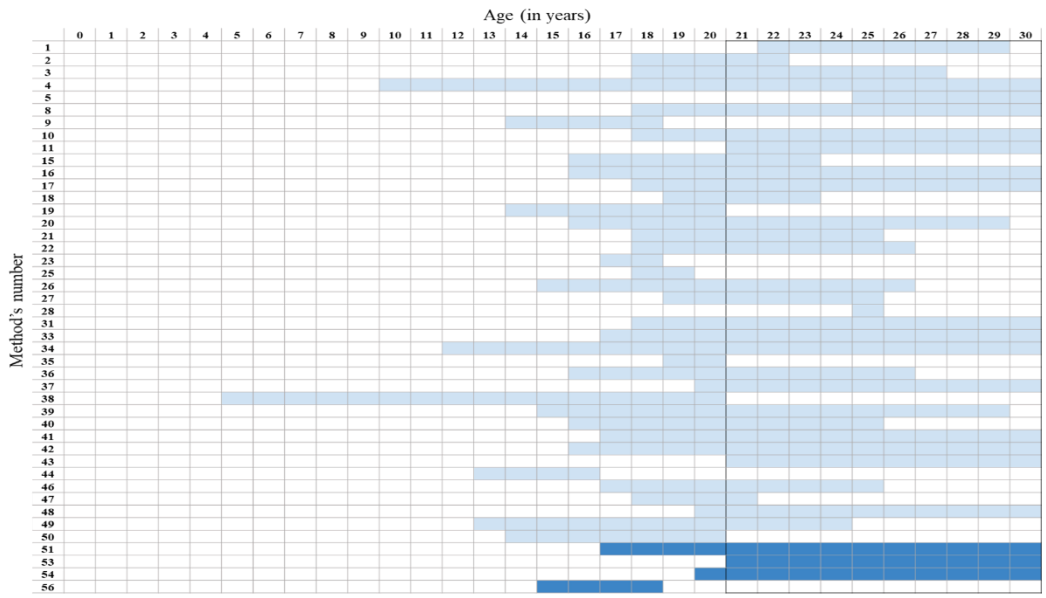

Figure 3.15.1.: *Diagram representing the AAD estimate provided by every method applied to individual [1406]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which every DD method overlap.*

From the initial fifty BD methods, a total of forty could be applied to this set of human skeletal remains, either due to the overall stages of BD and/or rates of preservation and conservation of the skeleton. From these forty methods, thirty-two gave an AAD estimate that coincided (either partially or

completely) with the estimate given by the DD methods, corresponding to a total of 80% of the methods applied. Hereon, the group composed by these thirty-two BD methods will be referred to as **Group_1406**. All the remaining methods underestimated AAD when compared to the DD methods. Age ranges provided by the methods from Group_1406 varied between a 1-year (method 28) and a 21-year interval (method 4), with an average of a 10.81-year interval (Figure 3.15.1.).

Group_1406 includes methods 1, 2, 3, 4, 5, 8, 10, 11, 15, 16, 17, 18, 20, 21, 22, 26, 27, 28, 31, 33, 34, 36, 37, 39, 40, 41, 42, 43, 46, 47, 48 and 49 (see Appendix A, Table A.25 for a correspondence between methods codes and their authors and year of publication).

Group_1406_Samples: A total of 43.8% (n=14) of the methods' samples are European, with half of these originating from Portugal (n=7). This was followed by a total of 28.1% (n=9) of Asian samples (six of these from Turkey), 9.4% (n=3) of African samples and 9.4% (n=3) of North American samples (Figure 3.15.2.). Samples' chronology ranged between the 19th (n=3) and the 21st Century, with more than half (n=18) being dated from the latter (Figure 3.15.3.B). SES was either described as "Low" (n=4), as "Low to middle" (n=8), as "Middle to high" (n=2) and "Low to high" (n=2). Half of these methods (n=16) either did not report or did not know its samples' SES (Figure 3.15.3.D).

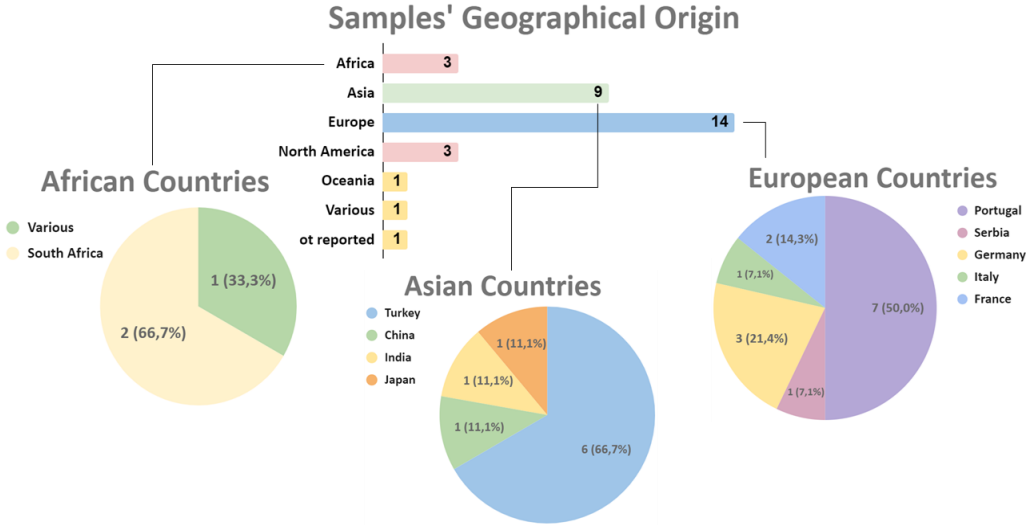

Figure 3.15.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_1406. In the centre bar graph the continent distribution is represented, followed by the pie charts of European, African, and Asian countries.*

Group_1406_Study materials: The most frequently used study materials were dry bone samples (n=12), followed by CT scan (n=8), X-ray (n=6), MRI (n=5) and human cadavers (n=1) samples (Figure 3.15.3.A).

Group_1406_Methodological approach: A total of 81.3% (n=26) of the methods used different stages of epiphyseal union to estimate AAD: the 3 stages of epiphyseal union were the most frequently used (n=8), followed by the 5 (n=9) and 8 (n=4) stages of epiphyseal union (Figure 3.15.3.E). The remaining six methods were based on the morphology of different skeletal elements, such as the sacrum (method 4), the medial clavicle (method 11), the pubic symphysis (method 22), the sternum (method 28) and a combination of different skeletal elements (methods 27 and 39) (Figure 3.15.3.C).

Group_1406_Anatomical areas: 68.8% (n=22) of the methods from Group_1406 were based on skeletal elements from the appendicular skeleton (Figure 3.15.4.): eleven methods were based on the lower limb (iliac crest: n=4; proximal tibia: n=1; pubic symphysis: n=1; distal femur: n=1; combination of different anatomical areas: n=4); ten methods were based on the upper limb (medial clavicle: n=6; proximal humerus: n=2; scapula: n=1; combination of different anatomical areas: n=1); one method was based on a combination of skeletal elements from the upper and lower limb (method 8). Eight methods were based on the axial skeleton (Figure 3.15.4.): one method was based on an anatomical area from the cranium (spheno-occipital synchondrosis: n=1); one method was based on the rib cage (sternum: n=1);

five methods were based on the vertebral column (presacral vertebrae: n=1; cervical vertebrae: n=2; sacrum: n=2). Only two methods used a combination of skeletal elements from the axial and appendicular skeleton (methods 1 and 5) (Figure 3.15.4.). A total 29.03% (n=9) of these used a combination of different skeletal elements to estimate AAD, while the remaining 70.97% (n=22) focused on only one.

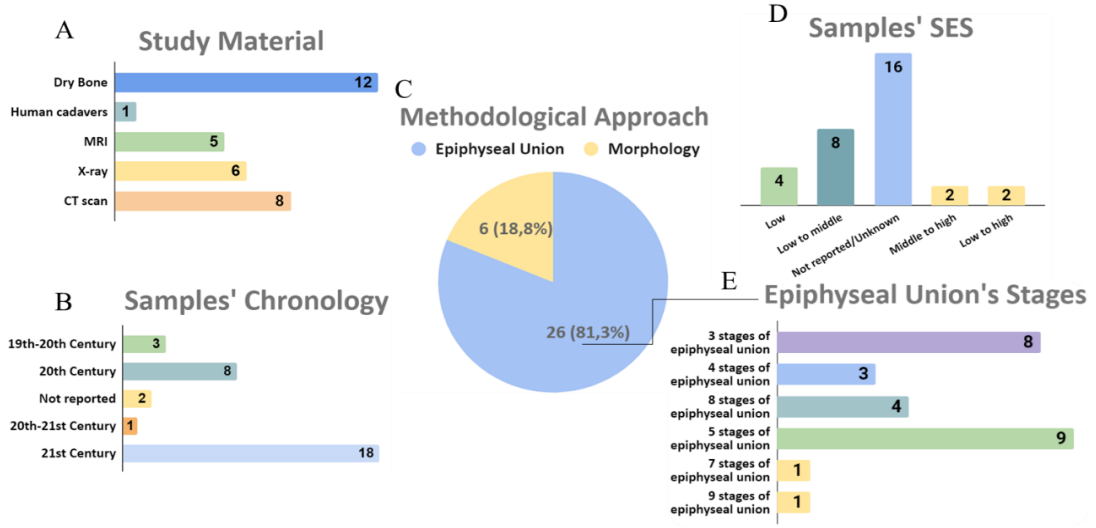

Figure 3.15.3.: *Charts regarding information about the BD methods from Group_1406: A – bar chart of the study materials used; B – bar chart of the samples' chronology; C –pie chart of the methodological approaches employed; D – bar chart of the samples' SES; E – bar chart regarding the stages of epiphyseal union used.*

Group_1406_Accuracy: From the total thirty-two methods from Group_1406, only five of these methods (methods 4, 22, 26, 28 and 39) reported values on accuracy. The other twenty-seven methods only provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.25).

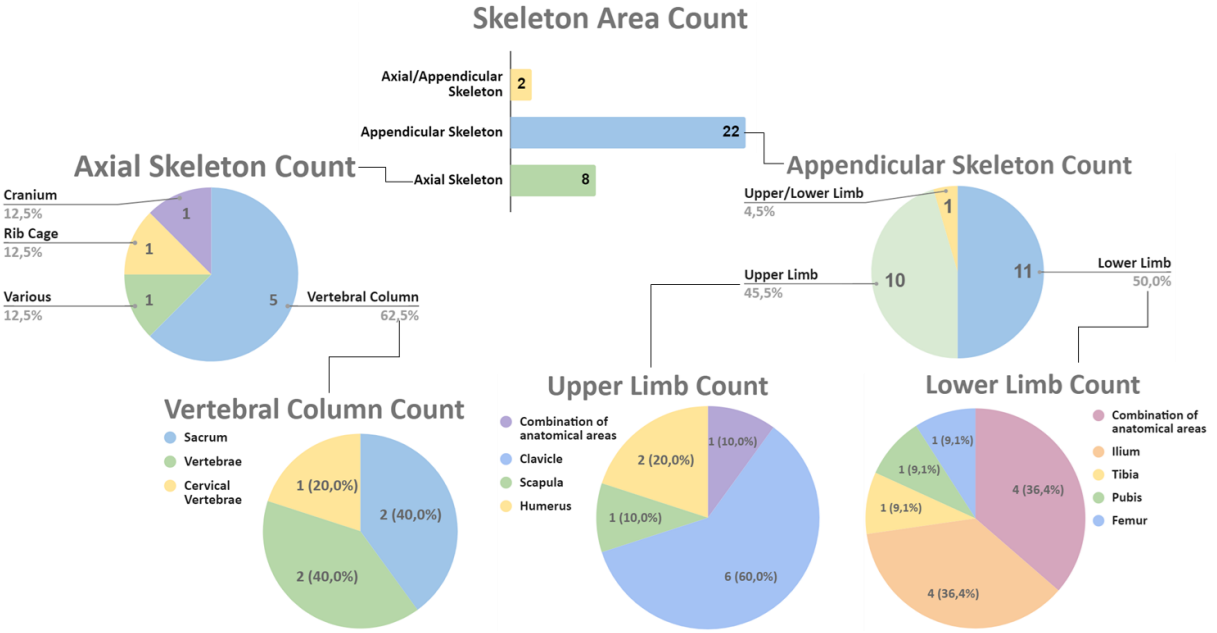

Figure 3.15.4.: *Bar graph and pie charts representing the frequency of each skeleton area used in the BD methods from Group_1406.*

1406_SIC: Three different types of SIC were identified in individual [1406] during analysis: LEH, CF and PR. LEH was observed in both maxillary and mandibular dentition, with higher incidence on the incisors and canines (every tooth presented one or more LEH). Premolars and molars also presented LEH, but with a smaller frequency. CF was observed bilaterally on the femurs' necks. PR was observed

across the periosteum of the long bones from the upper and lower limbs, mostly on the form of lamellar bone. Macroporosity and lytic bone lesions were also observed in bones such as the sternum and the sacrum.

3.16. [1419]

For individual [1419], an overall AAD estimate of 19-25 years was obtained, based on the reference BD methods (as previously explained, these methods correspond to Group_Dentition1 and were used as the reference methods in individuals without dentition preserved) (Figure 3.16.1.). Biological sex was estimated as male, based on the morphology of the os coxae and skull.

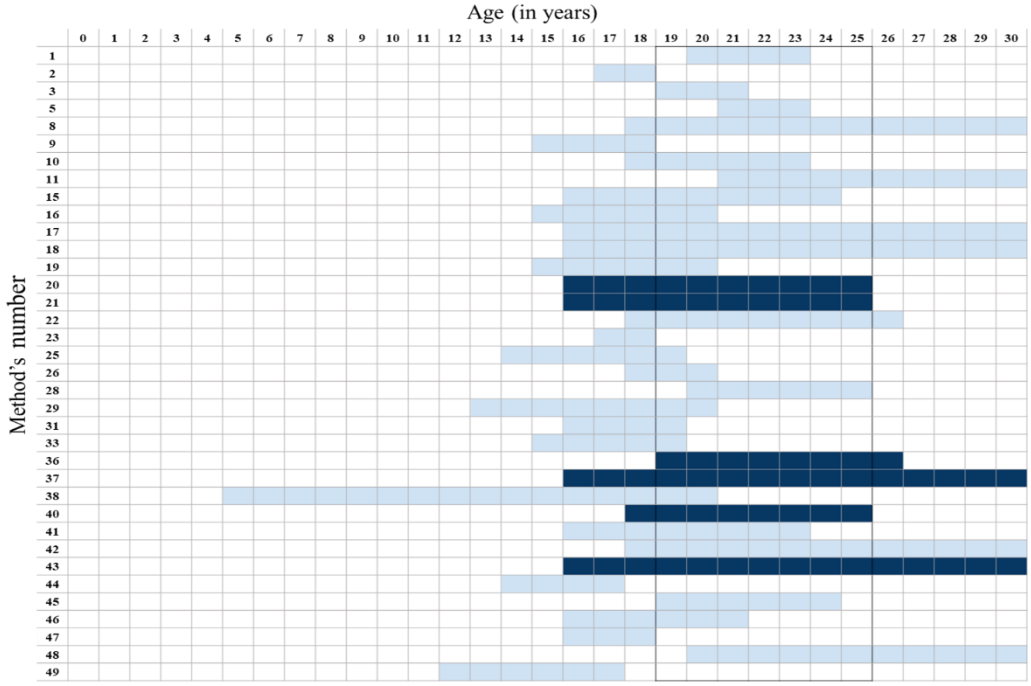

Figure 3.16.1.: *Diagram representing the AAD estimate provided by every method applied to individual [1419]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the reference BD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which the reference BD methods overlap.*

From the initial forty-four BD methods (the total fifty BD methods, minus the six BD methods used as reference in individuals without dentition – Chapter 3.3. for more details), only thirty could be applied to this set of human skeletal remains, either due to the overall stages of BD and/or rates of preservation and conservation of the skeleton. From these thirty methods, twenty-four gave an AAD estimate that coincided (either partially or completely) with the estimate given by the reference BD methods, corresponding to a total of 81.8% of the methods applied. Hereon, the group composed by these twenty-four BD methods will be referred to as **Group_1419**. All the remaining methods tended to underestimate age in relation to the reference methods. Age ranges provided by these methods varied between a 3-year (method 3, 5 and 26) and a 16-year interval (method 38), with an average 7.96-year interval (Figure 3.16.1.).

Group_1419 includes methods 1, 3, 5, 8, 10, 11, 15, 16, 17, 18, 19, 22, 25, 26, 28, 29, 31, 33, 38, 41, 42, 45, 46 and 48 (see Appendix A, Table A.26 for a correspondence between methods codes and their authors and year of publication).

Group_1419_Samples: 58.3% (n=14) of the methods' samples are European, with half (n=7) of these originating from Portugal; another 16.7% (n=4) of the samples are Asian, with half (n=2) of these from Turkey (Figure 3.16.2.).

Samples' chronology ranged between the 19th (n=2) and 21st Century, with more than half (n=13) dated from the current century (Figure 3.16.3.E). SES was either described as "Low to middle" (n=5), as "Low" (n=2), as "Middle to high" (n=2), as "Low to high" (n=1) or as "Middle" (n=1). The remaining twelve methods either did not have access or did not know the samples' SES (Figure 3.16.3.B).

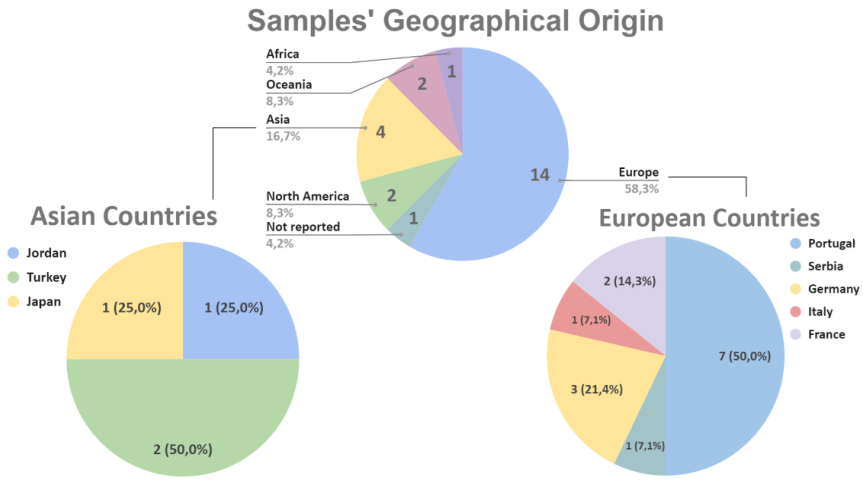

Figure 3.16.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_1419. In the centre pie chart, the continent distribution is represented, followed by the pie charts of European and Asian countries.*

Group_1419_Study_materials: The most frequently used study materials were dry bone samples (n=9), followed by CT scan (n=5), MRI (n=5), X-ray (n=4) and human cadavers (n=1) samples (Figure 3.16.3.A).

Group_1419_Methodological_approach: A total of 87.5% (n=21) of the methods used different stages of epiphyseal union to estimate AAD: the 3 stages of epiphyseal union were the most frequently used (n=8), followed by the 5 stages of epiphyseal union (n=5) (Figure 3.16.3.D). The remaining three methods were based on the morphology of different skeletal elements, such as the medial clavicle (method 11), the sternum (method 28) and the pubic symphysis (method 22) (Figure 3.16.3.C).

Group_1419_Anatomical areas: 75% (n=18) of the methods from Group_1419 used anatomical

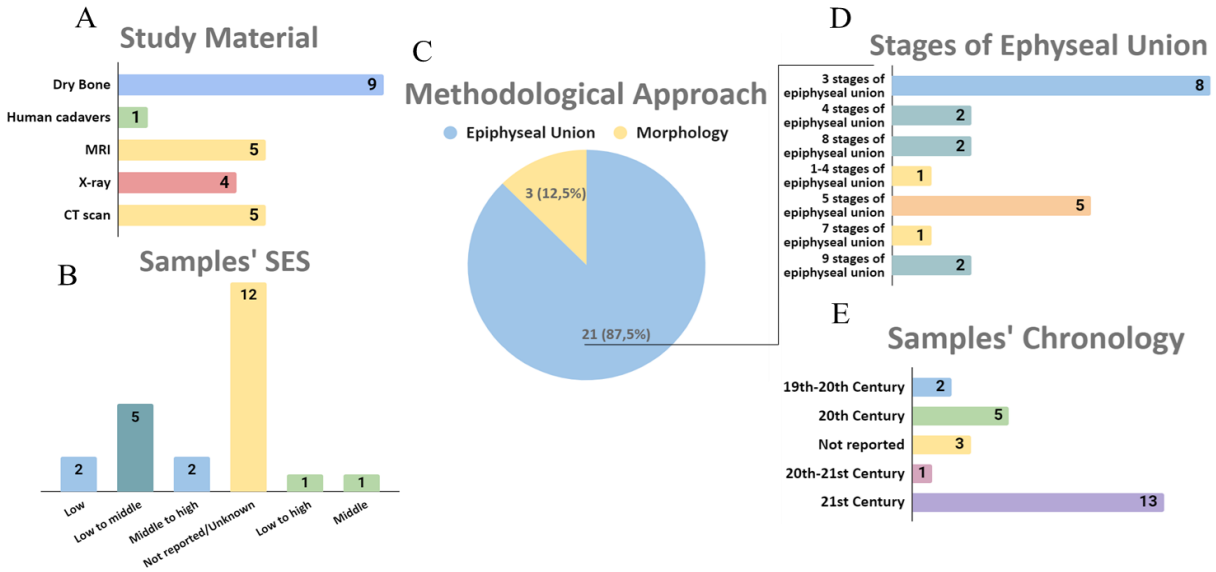

Figure 3.16.3.: *Charts regarding information about the BD methods from Group_1419: A – bar chart of the study materials used; B – bar chart of the samples' SES; C – pie chart of the methodological approaches employed; D –bar chart of the stages of epiphyseal union used; E – bar chart of the samples' chronology.*

regions from the appendicular skeleton (Figure 3.16.4.): nine methods were based on skeletal elements from the lower limb (iliac crest: n=2; pubic symphysis: n=1; distal femur: n=1; proximal tibia: n=2;

combination of skeletal elements: n=3); eight methods were based on the upper limb (medial clavicle: n=3; scapula: n=1; proximal humerus: n=1; combined skeletal elements: n=3); one method was based on a combination of the upper and lower limb (method 5). Four methods were based on the axial skeleton (Figure 3.16.4.): two were based on the vertebral column (presacral vertebrae: n=1; sacrum: n=1); two were based on the rib cage (sternum: n=2). Only two methods were based on a combination of skeletal elements from the axial and appendicular skeleton (methods 1 and 5) (Figure 3.16.4.). A total of 37.5% (n=9) of the methods used a combination of skeletal elements, while the remaining 62.5% (n=15) of the methods focusing on only one to provide an AAD estimate.

Group_1419_Accuracy: From the total twenty-four methods from Group_1419, only three reported values on accuracy (methods 22, 26 and 28). The other twenty-one methods only provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.26).

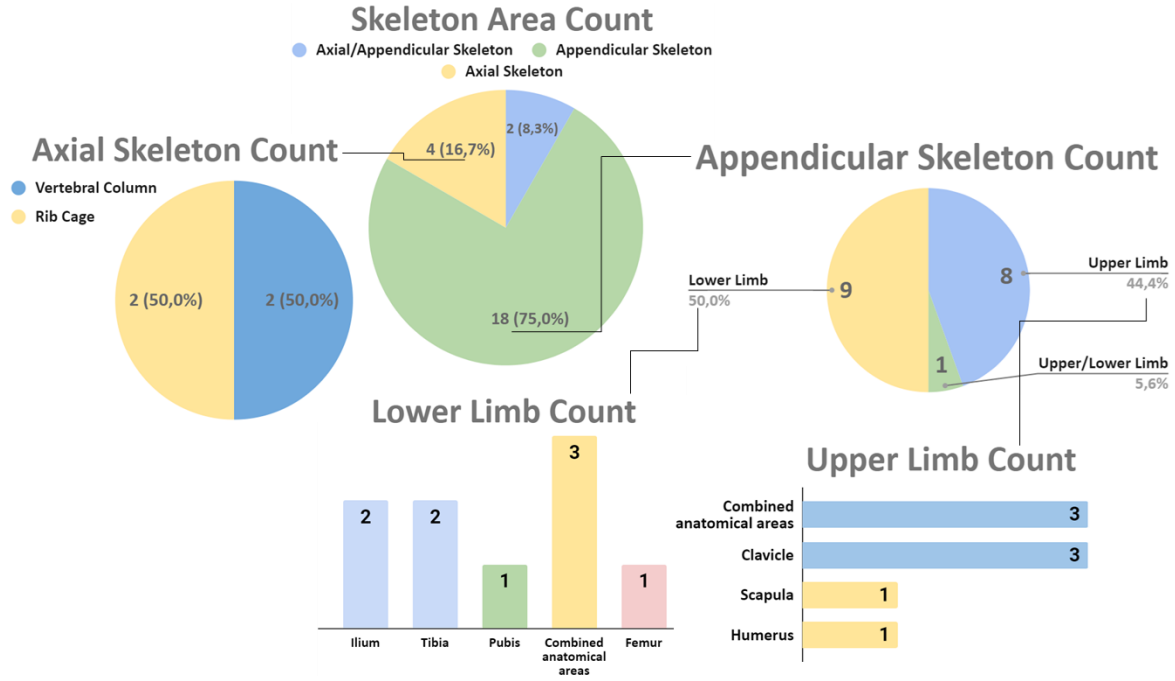

Figure 3.16.4.: *Pie charts and bar graphs representing the frequency of each skeleton area used in the BD methods from Group_1419.*

1419_SIC: Two different types of SIC were observed in individual [1419] during analysis: PH and PR. A possible case of PH was observed in various bones of the skull, most of them healed, with exception for the endocranial surface of the frontal bone, in which active microporosity was visible. PR was observed under the form of healed lesions and lamellar bone, mostly across the long bones' diaphysis from the upper and lower limb. A possible cloaca was also identified on the anterior view of the proximal humerus diaphysis.

3.17. [1429]

For individual [1429], an overall AAD estimate of >21 years was obtained based on DD methods' intersection (Figure 3.17.1.). Biological sex for this individual was estimated as female, based on the morphology of the os coxae and long bone measurements.

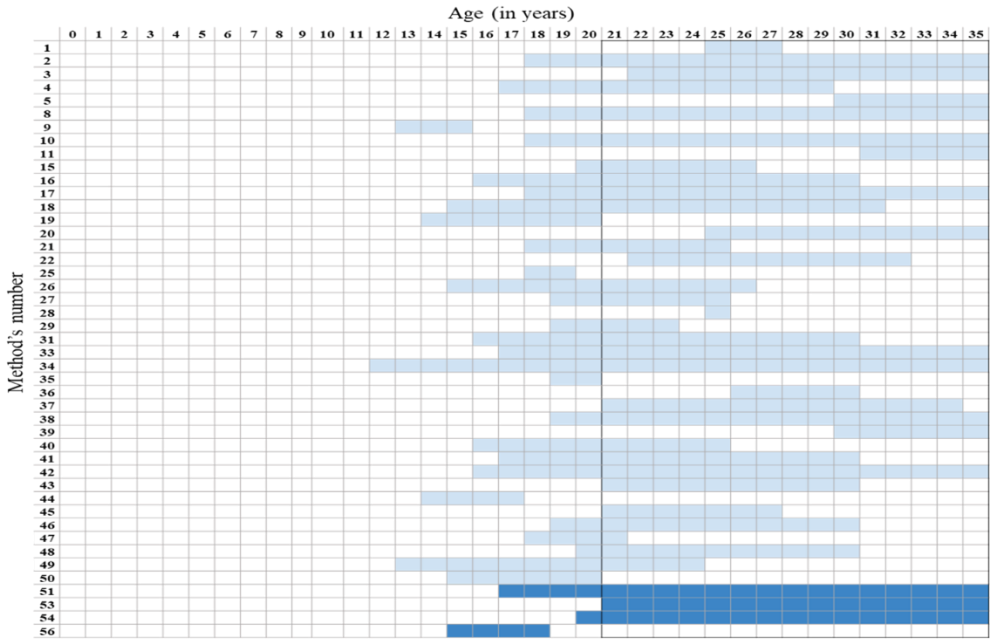

Figure 3.17.1.: *Diagram representing the AAD estimate provided by every method applied to individual [1429]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which every DD method overlap.*

From the initial fifty BD methods, only forty-one could be applied to this set of human skeletal remains, either due to the overall stages of BD and/or rates of preservation and conservation of the skeleton. From these forty-one methods, thirty-five gave an AAD estimate that coincided (either partially or completely) with the estimate given by the DD methods, corresponding to a total of 85.37% of the methods applied. Hereon, the group composed by these thirty-five BD methods will be referred to as **Group_1429**. All the remaining methods tended to underestimate age in relation to the DD methods. Age ranges provided by these methods varied between a 1-year (method 28) and a 24-year interval (method 34), with an average of a 11.91-year interval (Figure 3.17.1.).

Group_1429 includes methods 1, 2, 3, 4, 5, 8, 10, 11, 15, 16, 17 18, 20, 21, 22, 26, 27, 28, 29, 31, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 45, 46, 47, 48 and 49 (see Appendix A, Table A.27 for a correspondence between

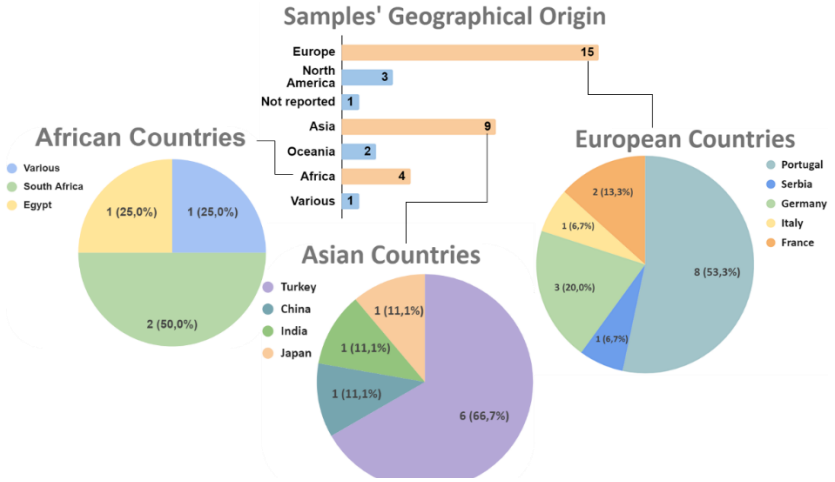

Figure 3.17.2.: *Scheme of the geographical distribution of the totality of AAD BD estimation methods from Group_1429. In the centre bar graph, the continent distribution is represented, followed by the pie charts of European, African, and Asian countries.*

methods codes and their authors and year of publication).

Group_1429_Samples: A total of 42.9% (n=15) of the methods' samples are European, with more than half (n=8) originating from Portugal; 25.7% (n=9) were Asian, most frequently from Turkey (n=6), and 11.4% (n=4) were African, most commonly from South Africa (n=2) (Figure 3.17.2.). Samples' chronology ranged between the 19th (n=3) and the 21st Century, with more than half (n=20) dated from the latter (Figure 3.17.3.C). SES was either described as "Low" (n=4), as "Low to middle" (n=8), as "Middle to high" (n=2) or as "Low to high" (n=2). The remaining nineteen methods either did not report or did not have access to the samples' SES (Figure 3.17.3.B).

Group_1429_Study_materials: The most frequently used study material were dry bone samples (n=13); this was followed by CT scan (n=9), X-ray (n=6), MRI (n=6) and human cadavers (n=1) samples (Figure 3.17.3.A).

Group_1429_Methodological_approach: A total of 82.9% (n=29) of the methods from Group_1429 were based on different stages of epiphyseal union: the 3 stages of epiphyseal union were the most used (n=10), followed by the 5 (n=9) and 8 (n=4) stages of epiphyseal union (Figure 3.17.3.E). The remaining six methods were based on the morphology of skeletal elements, such as the medial clavicle (method 11), the sternum (method 28), the sacrum (method 4), the auricular surface and/or pubic symphysis (methods 22 and 39) and a combination of the spheno-occipital synchondrosis, the third molar and cervical vertebrae (method 27) (Figure 3.17.3.D).

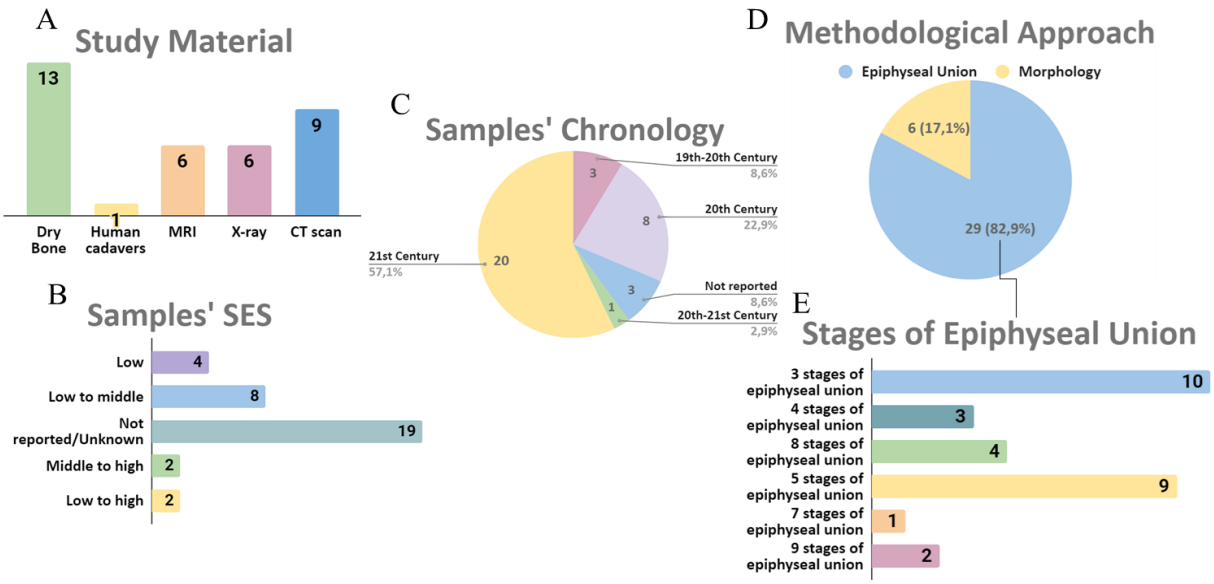

Figure 3.17.3.: *Charts regarding information about the BD methods from Group_1429: A – bar chart of the study materials used; B – bar chart of the samples' SES; C – pie chart of the samples' chronology; D – pie chart of the methodological approach employed; E – bar chart regarding the stages of epiphyseal union used.*

Group_1429_Anatomical areas: 68.6% (n=24) of the methods from Group_1429 were based on skeletal elements from the appendicular skeleton (Figure 3.17.4.): thirteen methods were based on the lower limb (iliac crest: n=4; proximal tibia: n=2; distal femur: n=1; pubic symphysis: n=1; combined anatomical areas: n=5); ten methods were based on the upper limb (medial clavicle: n=6; proximal humerus: n=2; scapula: n=1; combined anatomical areas: n=1); one was based on a combination of the upper and lower limb (method 8). Nine methods were based on the axial skeleton (Figure 3.17.4.): five methods were based on the vertebral column (cervical vertebrae: n=2; presacral vertebrae: n=1; sacrum: n=2); two methods were based on skeletal elements from the rib cage (sternum – methods 28 and 38); one method was based on the cranium (spheno-occipital synchondrosis – method 34); one method was based on the combination of spheno-occipital synchondrosis, third molar and cervical vertebrae (method 18). Only two methods used a combination of skeletal elements from the axial and appendicular skeleton (methods 1 and 5) (Figure 3.17.4).

A total of 30.3% (n=10) of the methods used a combination of skeletal elements, while the remaining 69.7% (n=23) of the methods focused on only one to estimate AAD.

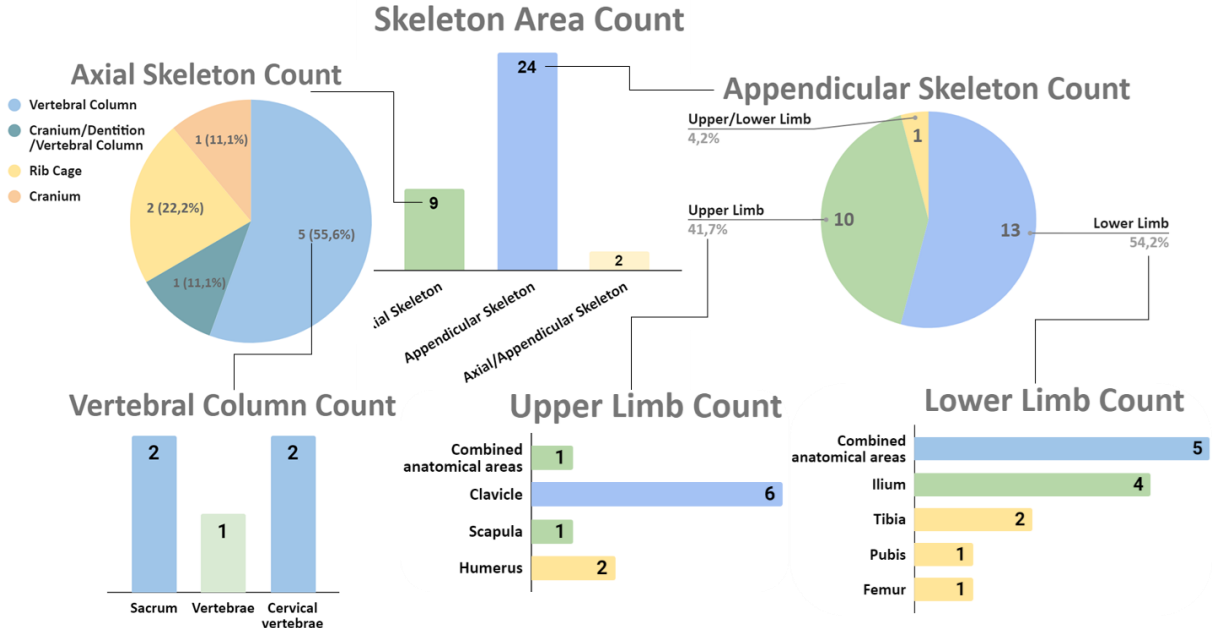

Figure 3.17.4.: *Pie charts and bar graphs representing the frequency of each skeleton area used in the BD methods from Group_1429.*

Group_1429_Accuracy: From the total thirty-five methods from Group_1429, only five methods (methods 4, 22, 26, 28 and 39) reported values on accuracy. The other thirty methods only provided values for intra- and inter-observer agreement and other parameters (for more detailed information and values, see Appendix A, Table A.27).

1429_SIC: Two types of SIC were identified in individual [1429] during analysis: LEH and PR. LEH were present on both maxillary and mandibular dentition, with higher incidence on the incisors and canines (at least one LEH per tooth). The premolars and molars also presented LEH, although to a lesser extent. PR was observed in the form of woven and lamellar bone, mainly across the diaphysis of the long bones, but also in bones such as the mandible, maxilla, and sinuses. Three pre-mortem fractures were also identified at different healing stages, in the medial right clavicle, and the intermediate section of the medial right humerus' diaphysis.

4. Discussion

The results obtained in this dissertation suggest that BD methods tend to underestimate AAD when compared to DD methods. This is shown by the fact that, in virtually every case presented, between one third and a half of the BD methods obtained lower values for AAD than DD methods. These results were observed in individuals with different maturational stages: from individuals without epiphyseal union commencement to individuals in which only late epiphyseal fusion (i.e.: medial clavicle, iliac crest and S1-S2) was observed.

The exceptions to this were individuals in which preservation and conservation rates were low, limiting significantly the amount of BD methods that could be applied. The methods that could be applied in these situations provided wider age ranges, which made the results more accurate but also less precise.

Another important finding was that, between individuals in which DD provided similar AAD estimates ([1214], [1296], [1314], [1406] and [1429]), the diagrams obtained from the BD methods were considerably different (Figure 4.1.). This means that the same BD methods gave different AAD estimates, even in cases where DD pointed to a similar maturational stage. This observation seems to support the idea that BD presents a higher degree of variability that DD, since individual variability was enough to influence AAD estimates based on BD, but not DD.

The next subsections of the present chapter will explore the results obtained previously, to understand why some of the BD methods consistently offered better results than the rest. Firstly, the dichotomy between geographical origin and SES followed by SES and SIC will be scrutinized, since these were the factors that offered the most interesting results. This will be followed by a discussion of other factors (such as samples' chronology, study materials used, anatomical regions and methods employed) that may be influencing each method's performance.

4.1. Geographical origin or SES?

In anthropological literature, two of the most studied factors that are thought to influence development rates and, consequently, reliability of AAD estimation methods, are the samples' geographical origin and SES.

The geographical origin principle defends that individuals from different parts of the globe would have different developmental rates due to environmental factors. In this case, populations from different continents, or even different countries, would develop at different times, requiring population-specific AAD estimation methods (Al-Qtaitat et al., 2016; Ekizoglu et al., 2015; Kotěrová et al., 2018; Marera & Satyapal, 2018; Sullivan et al., 2017). Even though developmental timings have been found to be distinct in different populations, the order in which these timings (such as the order of epiphyseal union across the skeleton) occur do not change between populations (Lenover & Šešelj, 2019). It is also important to point out that these timing differences were not always visible: in some cases, different populations would have similar growth rates. Hence, it should be questioned if the differences between populations are only due to geographical distance. This question becomes even more pertinent when observing the results obtained from the present dissertation.

The HRTS sample was most likely of Portuguese origin. Although most BD methods described as more reliable (i.e.: gave a similar AAD estimate to the reference methods in a higher number of times) in chapter 3. Results were based on Portuguese samples, a considerable amount of these methods had samples from several geographical regions. These included other European countries, but also countries from Africa and Asia, with Turkey as the second most common country of origin. These findings were

also observed in other studies (Cardoso, 2007; Frisancho et al., 1970a, 1970b; Schmeling et al., 2000, 2006b), suggesting that creating population-specific data will not necessarily be more beneficial or even more reliable.

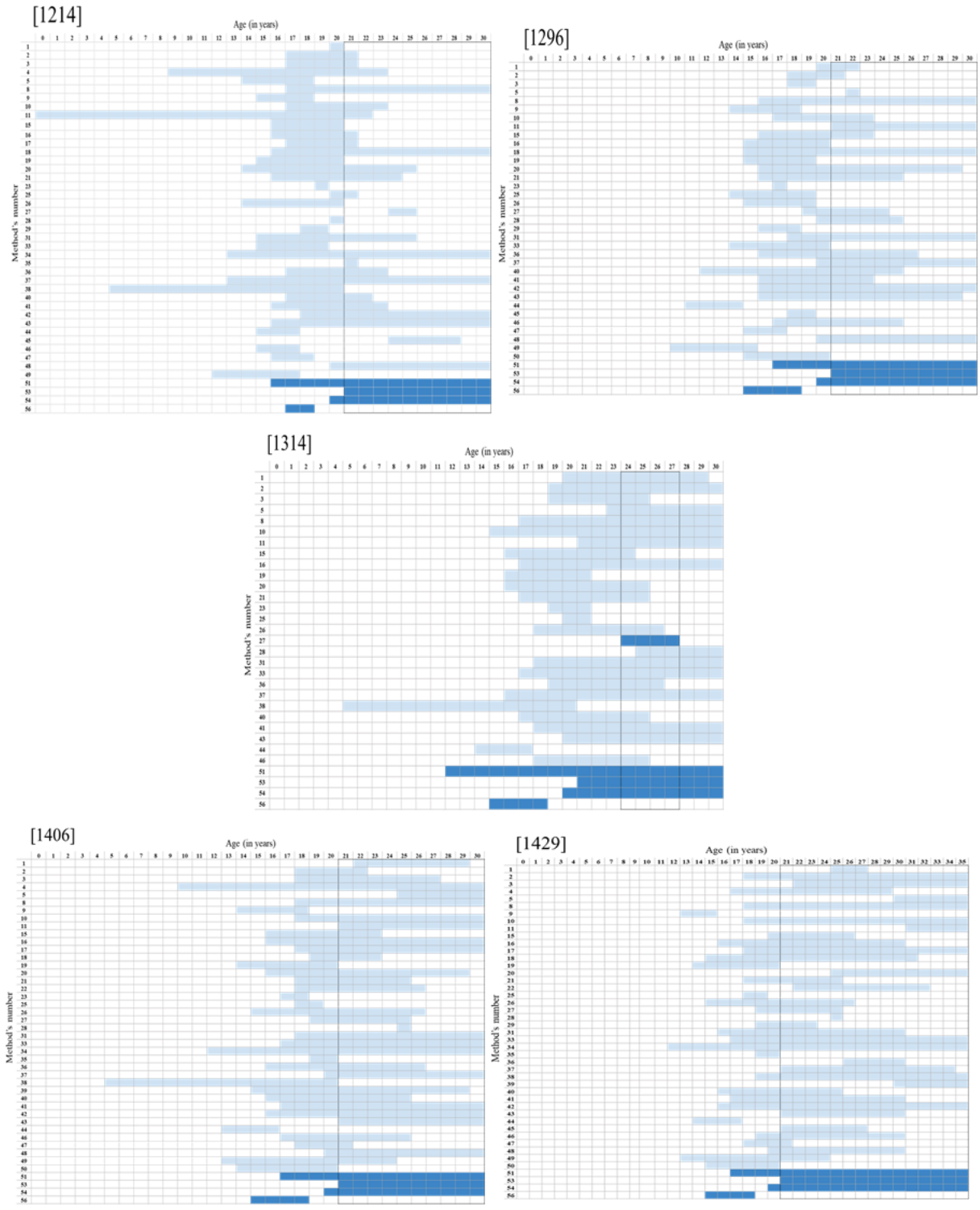

Figure 4.1.1.: Diagram representing the AAD estimate provided by every method applied to individuals [1214], [1296], [1314], [1406] and [1429]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which every DD method overlap.

The exceptions to these results are individuals without epiphyseal union ([575], [872] and [1008]) and individual [1313]. In these, the vast majority of methods used European samples, with only a small portion representing different geographical origins (see subchapters 3.8., 3.9., 3.10. and 3.13.)).

These different results in the three individuals without epiphyseal union may be explained by their general stage of development. Most AAD estimation methods available for these earlier stages of development are based on dry bone, hence, very commonly on identified osteological collections. These, in its turn, are much more numerous in European countries, especially in Portugal (Alves-Cardoso & Campanacho, 2022). Although [1313] is not included in the group of individuals without epiphyseal union, the low preservation and conservation rates did not allow the application of many BD methods, which may have influenced the results obtained from this individual.

Several authors still subscribe to the notion that geographical origin is essential when estimating AAD. This is based on the assumption that individuals from different geographical regions would have different developmental rates and when estimating age from dead or alive individuals, the methods chosen should have samples with similar origins (Al-Qtaitat et al., 2016; Boccone et al., 2010; Kocasarac et al., 2016; Kotěrová et al., 2018; Marera & Satyapal, 2018; Oldrini et al., 2016; Sullivan et al., 2017). The results obtained by the present dissertation seem to suggest that geographical origin might not be the most determinant factor, since the best performing methods have scattered geographical origins. To see what may be influencing these results, other factors are explored below.

SES is also another factor that has been studied with the aim of understanding its influence on developmental rates. SES is directly linked to each individual's access to proper health care, education and nutrition. When these criteria are subpart due to a low SES (common in low-income countries/regions), development may be directly affected (Al-Qtaitat et al., 2016; Cardoso, 2007; Cardoso et al., 2013a, 2013b, 2014; Cardoso & Ríos, 2011; Cardoso & Severino, 2010; Corron et al., 2019; Ekizoglu et al., 2019a, 2019b; Kocasarac et al., 2016; Marera & Satyapal, 2018; Oldrini et al., 2016; Passalacqua, 2013; Schaefer et al., 2015).

As described in chapter 1. Introduction, a correct nutrition is one of the main elements responsible for normal growth. When an individual undergoes under- or sub-nutrition during childhood, linear growth is interrupted to save energy for other essential systems. This can result in an individual's smaller height for age. If, during development, proper nutrition is restored, there is the possibility for a catch-up growth. If this is not the case, small height may become chronic, in a state known as stunting (Beaumont et al., 2018; Cardoso et al., 2013a, 2013b; Cardoso & Severino, 2010; El-Din et al., 2019; García-González et al., 2019; Passalacqua, 2013; Pérez et al., 2017; Primeau et al., 2016; Schaefer et al., 2015; Schmeling et al., 2006b). However, under- and/or sub nutrition do not affect only skeletal linear growth. Research conducted by different authors suggest that exposure to mal- and/or sub nutrition increases the likelihood of developing chronic diseases (such as osteoporosis, cardiovascular diseases, and diabetes), leaving individuals in higher states of frailty (Gluckman et al., 2005; Toole & Waldman, 1997).

Although SES has been studied as a factor capable of influencing human development, it is only a social marker reflecting the environmental conditions to which a person is exposed during development. A low SES tends to be connected to poor living conditions, and vice-versa. Poor living conditions have been proved to provoke a delay in development, suggesting that geography is not the main factor influencing development, but it is affected by SES. In fact, a low SES is frequently associated with some geographical areas, such as Africa and Asia, due to an unequal distribution of resources and limited access to proper food and healthcare, which lead to a higher rate of developmental delays and stunting in these populations (Cardoso, 2007; Frisancho et al., 1970a, 1970b; Schmeling et al., 2000, 2006b).

This is in agreement with the results obtained from the present dissertation. From the BD methods that gave a coincident AAD estimate to the reference methods, the most common SES descriptions were "Low" and "Low to middle". This may indicate that these samples faced social distresses that may have included under- and/or sub nutrition, impairing normal growth. SES from the HRTS is also estimated

as “Low”, based on the hospital’s patient’s history, and the general health, sanitary and food conditions found in Lisbon at that time (Alves-Cardoso et al., 2013; Assis et al., 2015; Ramos, 1993; Rijo, 2012), suggesting the possibility of similar developmental rates between the samples from the BD methods used and the HRTS sample.

It is not possible to overlook the amount of BD methods that do not report the samples’ SES status. Although most of these samples were from the 21st Century and these studies were based on living individuals, the fact that these individuals resorted to public health care and most of them did not use any health insurance may suggest a lower to middle SES. However, even information regarding the means to obtain the samples data is not provided in every method, making it impossible to assume that this is true in every case.

4.2. The relation between SIC and SES

As described above (see section 4.1. Geographical origin or SES?), SES can be considered a social marker for an individual’s access to health care, education, and proper nutrition during development, shedding light on the developmental problems that may be found in each individual. However, written information about the social context of each sample is not always available. This may be the case with archaeological samples, but also with forensic cases in which the individual’s life story is unknown.

In this case, biological markers of physiological stress may be useful (Beaumont et al., 2018; Mangas-Carrasco & López-Costas, 2021). These include various SIC, such as PH, CO, CF, CH, LEH and PR. Although there is a discussion about the possibility of some of these changes being individual characters (Chaichun et al., 2021), most of the studies about these SIC suggest that these have a direct link to an individual's state of health. This is the case for the known association of PH and CO to anaemia (Brickley, 2018; Chaichun et al., 2021; O’Donnell et al., 2020; Rinaldo et al., 2019). Although SIC have not been the focus of a great number of studies, leading to an uncertainty about the aetiological origin of most of these changes, these lesions have been associated with conditions of poor health, infectious diseases, and inadequate nutrition (Beaumont et al., 2018; Cardoso, 2007; Mangas-Carrasco & López-Costas, 2021; O’Donnell et al., 2020; Pilloud & Schwitalla, 2020; Towle & Irish, 2020). These factors have, in their own turn, been associated with a low SES and to developmental delays. Hence, an association between SES and SIC needs to be considered, allowing for a better understanding of an individual’s conditions during development when no written information is available.

In the HRTS sample, a high rate of SIC was observed, with every individual presenting various degrees of PR and LEH (the latter was only applicable to individuals in which dentition was preserved). The second most common type of SICs present were CF and CH. There were no individuals with visible CO and only [1419] presented a possible case on PH. The presence of these SIC is in agreement with the low SES that was estimated for this sample based on its social and historical contexts.

Through the results, it is observable that the individuals in which there were a higher degree of underestimation of AAD are also the individuals that presented a higher number of SIC ([575], [1008], [1214], [1296], [1406] and [1419]). This was especially true for individuals in generally earlier maturational stages: a total of forty-two BD methods were applied to individual [575], but only twenty methods complied with the AAD estimate provided by the DD methods; a total of twelve BD methods were applied to individual [1008], but only five overlapped with the AAD estimate provided by the DD methods; in both cases, less than half of the methods applied proved reliable when estimating AAD based on BD. Nevertheless, every individual showed IND discrepancies, even if in a smaller degree, and had at least one SIC observable during analysis.

It is relevant to note that none of the fifty BD methods applied to the HRTS sample presented a description of SIC assessment of the samples used to build each method. Only general gross pathological conditions were considered and excluded from the analysis. However, the results from the present dissertation suggest that age estimation requires SIC assessment, which is in its turn highly dependent on the environment in which the individual has developed and may be indicative of physiological stress during life and consequent developmental delays. AAD estimation methods need to consider an individual base assessment of development and pathological profile to have a more holistic view of the development conditions and how they may influence individual variation. An assessment of samples' SES and nutritional status is likely to increase methods' accuracy (Cardoso & Severino, 2010), to which assessment of SIC may serve as a biological marker of physiological stress.

Another important factor to consider is that the absence of SIC does not necessarily mean the absence of physiological stress during development. In these cases, it is important to consider the osteological paradox (Wood et al., 1992): if hardships are presented during life, a frailer person may not survive long enough for stress markers to develop on the skeleton, since this is a slow and nonspecific response. On the other hand, an individual that presents a high degree of SIC active at time of death, may be more resistant to strenuous conditions, surviving long enough through physiological stress for SIC to develop. Hence the importance of social context: the presence or absence of biological markers, when considered alone, may have disparate interpretations that do not necessarily correspond to reality (Cardoso, 2007; Cunha et al., 2009; Mangas-Carrasco & López-Costas, 2021; Primeau et al., 2016; Schmeling et al., 2006b). It is by pooling biological, social and environmental information about the sample that the investigator can obtain an image closer to the reality of those individual's lives. Without this, the stagnant image obtained by studying the sample in either one or the other domain will always be subpart.

This also leads to another important question: is the search for a sample in perfect health conditions realistic? As previously mentioned, most of the individuals in need of age estimation, whether living or dead, whether in an archaeological or forensic contexts, disproportionately tend to originate from less favourable environments. This means that there is a higher chance that the individuals in need of age estimation come from a low to medium SES and of presenting SIC and developmental delays. In this case, using methods of AAD estimation that were based on samples with optimum environmental conditions during development may cause an underestimation of age of these individuals. The exclusion of individuals with pathological changes and/or presence of SIC from samples used to build AAD estimation methods should be questioned. This is even more important in cases where information regarding samples' SES and/or SIC assessment is completely absent, since geographical origin does not seem to provide enough information to decide which AAD estimation method should be used.

4.3. Other factors that should be considered:

4.3.1. Chronology

Even though the HRTS sample is dated from the first half of the 18th Century (Alves-Cardoso et al., 2013), the BD methods that gave a similar AAD estimate to the reference methods in a higher number of times (see "chapter 4: Results" for a more detailed description) are dated between the 19th and the 21st Century. The only exceptions to these findings are the individuals without epiphyseal union ([575], [872] and [1008]) and individual [1313], in which the methods' samples have chronologies ranging between 2300-1750 BCE and the 21st Century.

With exception to the above-mentioned cases, around half of the BD methods discussed in the previous chapter have samples dated from the 21st Century. This appears to suggest that, even though

evidence of a secular growth trend has been found in modern populations (Fudvoye & Parent, 2017), it does not seem to affect the timings of epiphyseal union. In this sense, if the AAD estimation methods used are based on the epiphyseal union timings, samples' chronology may not be a decisive factor when choosing the method.

Younger individuals ([575], [872] and [1008]) showed different results: the methods with the higher number of similar AAD estimates to the reference methods used in each individual used samples dated between 2300-1750 BCE and the 20th Century. These findings may be explained by two different factors.

The first factor, described by Cardoso (2007), suggests that growth retardation influenced by environmental constraints is more prominent in younger individuals. This is because it would mainly affect the long bones' diaphysis linear growth, while epiphyseal union would be less affected by these same circumstances. Hence, younger individuals from the HRTS sample that were more affected by strenuous environmental conditions could have less similarities to contemporary samples with the same age range, but that were exposed to better living conditions. On the other hand, older individuals could present more resemblances to contemporary samples despite the different environmental conditions they were subjected to during development, due to a similar timing of late epiphyseal fusion (such as medial clavicle, iliac crest and S1-S2 fusion). This is a plausible explanation for why methods based on archaeological samples gave more reliable AAD estimates in individuals at earlier stages of development and why methods with samples dated from the 21st Century, based on the epiphyseal union, gave better AAD estimates in individuals at later stages of development.

The second factor may be the absence of methods with samples of individuals at earlier development stages, that are dated from the 21st Century. This means that the number of studies performed in individuals at earlier development stages in contemporary samples is limited. Studies *in vivo* in individuals at earlier developmental stages have several ethical questions, specifically regarding informed consent that cannot be given by an infant or child. This may lead to a deficiency in the number of methods based on modern samples of younger individuals, which could also explain why methods with samples from previous centuries provided better results in these cases. Where *in vivo* studies fail to fill an important blank in AAD estimation, osteological collections have the potential to thrive. Hence why most of the methods that conveyed a similar AAD estimate to the reference methods were based on dry bone samples. This still presents as a difficulty, because, even though these samples could be more accessible for study, infant and children's bones have lower preservation rates due to their fragility and to inexperience in the field during excavation, which may leave smaller bones unexhumed (Boccone et al., 2010; Olivares & Aguilera, 2017; Pérez et al., 2017), leading to smaller samples than what would be ideal to provide AAD estimation standards. However, osteological collections are still the best option for constituting AAD estimation methods in younger samples, when considering accessibility and ethical issues.

4.3.2. Sample size and sex and age distribution

The individual results described in chapter 3. Results show a slightly higher sample number average, when compared to the average sample size of the fifty BD methods in total. This means that the BD methods that gave a coincident age estimate to the reference methods were tendentially the ones composed by larger samples. In both cases, the gap between males and females is present, showing an overrepresentation of male individuals in the samples.

Sample size, sex and age distribution are important parameters that can be determinant for the general statistics employed in each method. For example, a small sample (Al-Qtaitat et al., 2016; Campanacho et al., 2018; Cardoso, 2008a, 2008b; Cardoso et al., 2013b, 2016; Cardoso & Severino,

2010; Coqueugniot & Weaver, 2007; Olivares & Aguilera, 2017; Kocasarac et al., 2016; Milenkovic et al., 2013; Passalacqua, 2013; Pérez et al., 2017; Singh et al., 2020) limits the amount of individual variability included in the method. If several age categories are constituted by as little as one or even zero individuals (for example: Boccone et al., 2010; Cardoso, 2008a, 2008b; Cardoso & Ríos, 2011; Cardoso & Severino, 2010; Coqueugniot & Weaver, 2007; Passalacqua, 2009, 2013), some consideration may be needed when applying these methods, since they do not include a sufficient number of individuals to include enough individual variability and are mainly based on statistical work. This means that there may be a higher chance of giving a wrong AAD estimate: one individual that has a certain chronological age category does not represent the entire possible spectrum of developmental stages that can be found at that chronological age.

Sex distribution is also a relevant factor for AAD estimation. Although differences between male and females are not as visible in the early stages of development (Cardoso et al., 2014; Coussens et al., 2002), these differences seem to increase around puberty, with the development of sexual dimorphic characteristics. However, as described above, male representation is more significant than female representation in most of the AAD estimation methods (Cardoso et al., 2016; Dudzik & Langley, 2015; El-Din et al., 2019; Gurses & Altinsoy, 2021; Hocaoglu et al., 2020; Olivares & Aguilera, 2017; Milenkovic et al., 2013; Oldrini et al., 2016; Passalacqua, 2009; Torimitsu et al., 2019). Since females tend to enter puberty at a younger chronological age than males (Cunha et al., 2009; Frisancho et al., 1970a, 1970b; Schmeling et al., 2006b), this may result in an underestimation of females' chronological age. Even if methods consider males and females separately, a smaller percentage of females implies a lower representation of individual variability, still increasing the margin of error for AAD estimation when it comes to female individuals during development.

Age intervals are also important, especially when considered together with age distribution. When dividing an age range into different age categories, every category should have a similar number of individuals (male and female) in order to optimise the results. For example, if a sample includes an age range between 0 and 30 years, but more than half of the samples is equal to or older than 20 years, the statistical results will tendentially classify the individuals as older than their real chronological age. This happens because these are the age categories that will include more individual variability (Dudzik & Langley, 2015; García-González et al., 2019; Hillewig et al., 2013; Schaefer et al., 2015).

These factors may be influencing the results obtained, explaining in part why most of the methods tended to underestimate the age of the HRTS sample. Yet, some of the methods with the best performance still have small samples (methods 1, 2, 7, 8, 10, 11, 12, 17, 23 and 42, all with samples under 200 individuals – for a correspondence between methods' codes with authors and year of publication, see Table 2.1.1.), with unequal sex and age distribution.

4.3.3. Dataset used in method development

Around half of the fifty BD methods used dry bone samples as study material. The remaining methods used CT scans, X-rays, MRIs and human cadavers' samples. The results obtained also show that methods based on dry bone samples offered the most reliable AAD estimate both in individuals and in grouped samples.

Even though methods based on X-ray and CT scan obtained good results in the individual analysis, in the pooled analysis the same did not happen. Methods using these medical exams offered the best results in the individual analysis, after the methods based on dry bone samples. However, when pooling the results from the individuals (subchapters 3.3., 3.4., 3.5. and 3.6.) these methods did not show up as the ones giving the most reliable AAD estimates. This suggests that the methods based on dry bone samples were the same in most of the cases, while methods based on CT scan and X-ray that gave a

coincident AAD estimate to the reference methods were different in each individual (i.e.: each of these methods gave a reliable AAD in a small number of individuals).

These results seem to support the notion than the AAD estimation methods chosen should preferably be based on a similar study material to the one being analysed, since some details observable in dry bone may not be noticeable in different types of medical examinations, or vice-versa (Alhazmi et al., 2017; Cardoso et al., 2013a; Cardoso & Severino, 2010; Chowdhuri et al., 2018; Ekizoglu et al., 2019a, 2019b; El-Din et al., 2019; Marera & Satyapal, 2018; Nougarolis et al., 2017; Primeau et al., 2016).

4.3.4. Anatomical elements used to build AAD methods.

Most of the fifty BD methods applied to the HRTS sample were based on skeletal elements from the appendicular skeleton. From these, the skeletal elements from the lower limb were the most common. More than half of the methods also focused on only one skeletal element instead of a combination of different elements. In general, the most frequent anatomical areas used to build AAD methods were the medial clavicle and the iliac crest.

The higher number of AAD estimation methods based on these anatomical areas may be linked to current necessities of age estimation, especially on living individuals seeking refuge. Since these two epiphyses are of late fusion, they are commonly used to determine the probability of having attained the threshold of 18 years of age. The iliac crest and the medial clavicle are also easily accessible by medical examinations, such as X-rays, CT scans and MRIs, which are the main study material of the methods that focus on these skeletal elements. Hence, why there is a relatively recent interest in these anatomical areas for building age estimation methods (Chowdhuri et al., 2018; Ekizoglu et al., 2015, 2019a, 2019b; Hillewig et al., 2013; Krämer et al., 2014; Lottering et al., 2017; Mahon et al., 2018; Milenkovic et al., 2013; Schaefer et al., 2015; Torimitsu et al., 2019; Wittschieber et al., 2013; Zhang et al., 2015).

The results show that methods based on the appendicular skeleton showed the best results in every case (see chapter 3. Results). This was true whether these individuals were in a more initial stage or reaching the end point of skeletal development.

Most of the methods that gave a coincident AAD estimate to the reference methods in a higher number of times are based either on the medial clavicle or the iliac crest, suggesting there is more uniformity (less individual variability) in the timing of epiphyseal union in these two anatomical sites.

The results obtained in the group of individuals without dentition preserved show a higher reliability on methods based on the lower limb. The absence of methods based on the upper limb in this group may be justified with the lower degree of conservation and preservation from this group's individuals. This may suggest that in samples with a low conservation and preservation rates, methods based on a single skeletal element from the lower limb may be more accurate when estimating AAD, since these bones are more resistant to taphonomic changes.

In the group of individuals without epiphyseal union, most BD methods were based on measurements of the long bones from the upper and/or lower limb, suggesting a better performance of methods based on long bone measurements in individuals with earlier developmental stages (i.e.: in which epiphyseal union has not yet commenced or is in early stages).

The fact that only a small number of BD methods used a combination of skeletal elements is also suggestive that, in individuals in which the epiphyseal union is partial/complete, the methods with higher reliability are those based on a single skeletal element, either from the upper or the lower limb.

4.3.5. Methodological Approach

Methods' methodological approach was either based on morphology, measurements, or epiphyseal union of different skeletal elements, with the latter being the most common approach. In individuals at earlier developmental stages, results suggest that measurements may provide a more reliable methodological approach for AAD estimation, since no epiphyseal union has commenced. In individuals [505], [872] and [1008], the methods based on epiphyseal union were the ones that gave wider age intervals, up to a 20-year interval (individual [1008], method 38), which does not offer enough precision for a useful AAD estimate.

It is also observable that, besides epiphyseal union, in the individuals with partial/complete epiphyseal union (at later developmental stages), the remaining methods that gave a more reliable AAD estimate were based on the morphology of different anatomical regions. In individuals at later stages of skeletal development, measurements are more variable due to sexual dimorphism and individual variation. The results suggest that, in these cases, AAD estimate are more reliable when using methodological approaches such as the epiphyseal union and/or morphology. In these cases, sex-specific methodology should be considered (Alhazmi et al., 2017; Al-Qtaitat et al., 2016; Ekizoglu et al., 2015; El-Din et al., 2019; Gurses & Altinsoy, 2021; Krämer et al., 2014; Lottering et al., 2017; Milenkovic et al., 2013; Primeau et al., 2016; Schaefer et al., 2015; Sullivan et al., 2017).

Regarding staging of epiphyseal union in the individuals in later developmental stages, the most commonly used were the 5 stages, 3 stages and 8 stages of epiphyseal union. However, it is also important to notice that these methods, especially those based on the 3-stages of epiphyseal union, are the ones that provided wider age ranges. In the individuals in earlier developmental stages, ([872] and [1008]), the most common methodological approaches were the 3-stages, 4-stages, and 6-stages of epiphyseal union.

The results from the pooled samples seem to reinforce the individual results. For individuals in an earlier developmental stage, the methodological approach that appears to be more reliable is the measurements of the long bones. If, due to conservation or preservation, these are not possible to apply, then methods based on the 4 stages and the 6 stages of epiphyseal union, as described by Cardoso et al. (2013) and Cardoso et al. (2014) respectively, seem to achieve reliable results as well.

On the other end, in individuals in later developmental stages, epiphyseal union seems to provide better results. This is especially the case when using the 3 stages, 5 stages or 8 stages of epiphyseal union, as described by Cardoso (2008a, 2008b), Kellinghaus et al., (2010) and Schmeling et al. (2004) respectively. When these methods cannot be applied to the sample, either due to preservation/conservation rates or to the finalisation of some of these epiphyseal union, the methods based on the morphology of the medial clavicle and the sternum (methods 11 and 28) also seem to convey reliable results.

However, even though the methods using the 3 stages of epiphyseal union were the ones with the best results, these are also the ones that gave wider age ranges. Since the 3 stages divide epiphyseal union in "no fusion", "partial fusion" and "complete fusion", the age ranges provided are broad. The category with the most helpful age estimate is the "partial fusion", since this conveys a lower and upper limit for age range, while the stages of "no fusion" and "complete fusion" only provides an upper and lower age limit, respectively (Cardoso, 2008a, 2008b). A clear example is the case of method 2, in which the range for the stage of "no fusion" for the clavicle is from birth to 20 years of age. This is because 20 years was the age of the oldest individual from the sample used by the author in which there were no signs of epiphyseal fusion. Similarly, the existence of one single category designated as "partial fusion" instead of several subcategories, as in the descriptions made by Kellinghaus et al. (2010) and Schmeling et al. (2004), provides wider ranges when estimating AAD. This means that, even though methods based on the 3 stages of epiphyseal union tend to be more accurate, they also tend to be less precise. Even

though creating substages for every phase (particularly the "partial fusion" phase) may be more useful for establishing smaller age ranges, (as is done in methods using 5 or 8 stages for epiphyseal union), there is a risk of increasing precision with a consequent decrease in accuracy. When applying these methods, professional experience of the anthropologist should help to determine which is the most appropriate methodological approach to use.

4.3.6. Results vs Accuracy

Even though Accuracy is one of the most important parameters to evaluate an AAD estimation method reliability, only six BD methods out of the total fifty reported these values. This suggests that only those six BD methods used at least one validation sample. To confirm the results obtained from the sample under study, the standards obtained must be tested in other samples to see if the method is accurate and reliable. However, this did not happen in most of the methods used, either due to constraints on time or the availability of a validation sample (Cardoso et al., 2016).

Accuracy reported by these six methods is very variable, with values ranging between 13% to 100%. A reasonable assumption would be that methods with higher accuracy would present better results in the samples to which they are applied. However, the results from the present dissertation show the opposite. In the pooled samples (see subchapters 3.2., 3.3., 3.4., 3.5. and 3.6.) the only methods with accuracy reported are methods 26 and 28, with values between k=0.90-0.95 and 13%-88%, respectively. There is a clear absence of the methods with accuracy reported as being >95% (methods 4, 22 and 44).

These differences may be justified by the statistical tests used in each method, but it may also be caused by the samples themselves. The fact that methods with higher accuracy did not perform well in the HRTS sample, but methods with lower accuracy did may mean that the samples initially studied to form the AAD methods may have more in common (in terms of development rate) to the HRTS sample than to the sample used to validate the methods' results. If this would be the case, methods' accuracy could differ depending on the samples used. Considering this as a possible explanation, it would be important to understand what similarities and differences exist between the samples that could influence the AAD estimation method reliability.

However, even the BD methods that had given a similar AAD estimate to the reference methods the most times (independently of the accuracy reported) presented relatively wide age ranges. Again, these results reinforce the notion that the more accurate methods also tend to give less precise results (i.e.: wider age ranges).

4.3.7. Difficulties frequently reported in the BD methods

Some of the most frequently reported shortcomings of the BD methods are the small sized samples and the unequal sex and age distributions, which influence the statistical results obtained. Another difficulty is the lack of standardisation regarding the methodology and statistical methods used, which impairs comparisons between different methods to test which could be more accurate and reliable (Cardoso & Ríos, 2011; Ekizoglu et al., 2019a, 2019b; García-González et al., 2019; Gurses & Altinsoy, 2021; Kocasarac et al., 2016; Milenkovic et al., 2013; Oldrini et al., 2016; Torimitsu et al., 2019).

The problems of small sample sizes and lack of information about the sample's social environment and clinical history have been reported for over 20 years (Schmeling et al., 2000, 2006b, 2007), but these difficulties have yet to be overcome.

Most of the BD methods are also qualitative, with only a small percentage using a quantitative approach (Dudzik & Langley, 2015; García-González et al., 2019). Both approaches have their

advantages and disadvantages. When using a qualitative approach, there is the advantage of being a more individual-based approach and is more dependent on the anthropologist experience, but this also means that this is a more subjective approach. On the other hand, when using a qualitative approach towards AAD estimation, the analysis becomes more objective, but it also has less consideration for individual variation.

Even though a more objective methodology may have its advantages for AAD estimation, by facilitating comparisons between different methods and being less dependent on professional experience, individual variation is still a very important factor and should not be disregarded. A good example of this situation is the case of individuals [1296], [573] and [1419] and the AAD estimate given by methods 2 and 3 (Figure 4.3.1.). Methods 2 and 3 were made by the same author, in the same year, using similar samples from the Lisbon Identified Human Skeletal Collection, with similar age and sex distributions and similar SES. Method 2 was based on the skeletal elements from the lower limb, and method 3 based on skeletal elements from the upper limb. Although in most of the cases the AAD estimates given by these two methods were similar, this was not the case for these three individuals. In [1296], age range provided by DD methods was 21 years or older. Method 2 gave an age range of 18-21 years and method 3 of 18-19 years. In [573], the same results were obtained: the age range provided by the reference BD methods was 20-25 years, to which only method 2 complied, with an AAD estimation between 18-22 years (method 3 gave an age range of 18-19 years). In [1419], the results were inverted: the age range provided by the reference BD methods was of 19-25 years, to which only method 3 gave an overlapping age estimate of 19-21 years (method 2 gave an age estimate of 17-18).

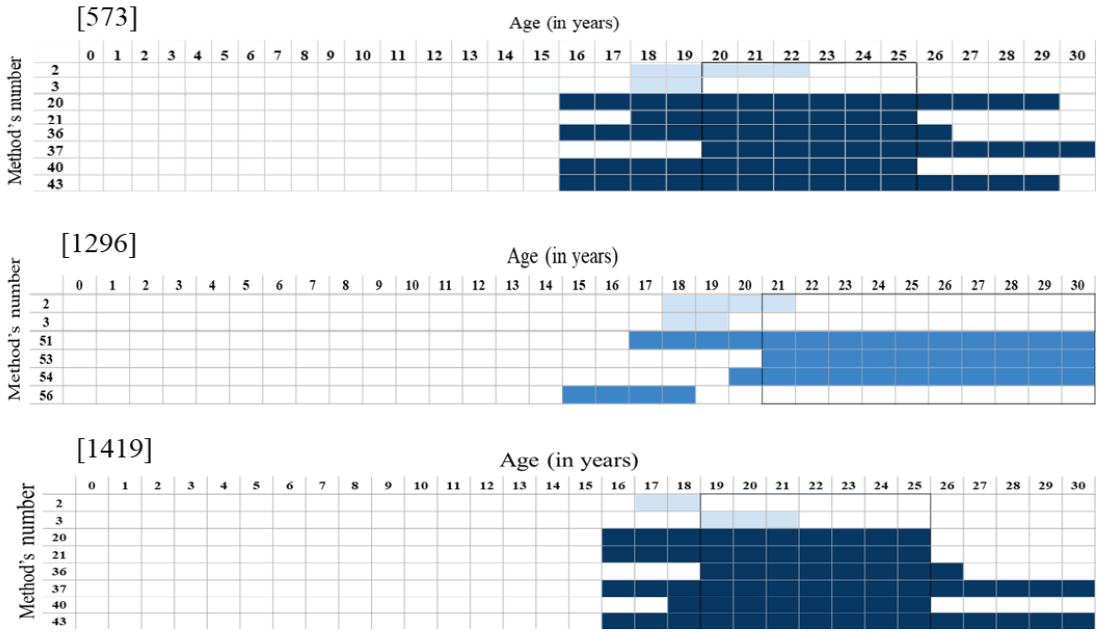

Figure 4.3.1.: *Diagrams representing the AAD estimate provided by methods 2 and 3 in individuals [573], [1296] and [1419]. In the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the reference BD methods (in individuals [573] and [1419]) and from the DD methods (in individual [1296]) are represented by the bars in darker blue, and the bars in light blue represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which the reference BD methods overlap.*

These findings highlight two important points. The first one is that individual variation must be considered, as even the use of AAD estimation methods based on the same sample may produce different results in one individual. The cases described above suggest that those three individuals had different rates of development between the upper and lower limbs, when compared to the samples from the Lisbon Identified Human Skeletal Collection, which is of similar chronology and SES. The pooled sample of individuals with partial/complete epiphyseal union (see subchapter 3.5.) suggests that methods based on the lower limb tended to be more reliable in the HRTS sample. However, in individual [1419] it is the

method based on the upper limb that gives a more reliable age estimate, stressing the importance of considering intra-population variation.

The second point is that, in the three cases described, it was the method that gave a wider age range that coincided with the AAD estimation provided by the reference methods. This means that, even though method 2 in [573] and [1296] and method 3 in [1419] were less precise (gave wider age ranges), these were also more accurate (gave more reliable age estimates). It also explains why AAD estimation methods cannot produce exact chronological age estimates based on biological age. It should always be provided as a probable age range (Dudzik & Langley, 2015; Hillewig et al., 2013).

Another interesting approach that should be taken into account is the Minimum Age Principle (El-Din et al., 2019; Gurses & Altinsoy, 2021), in which the estimated age range has as the lower value the minimum age possible for that particular stage of development, instead of a "more probable" range (for example, when using 95% or 68% confidence intervals). Even though this may cause a slight decrease in precision due to the wider age ranges provided, it allows for a higher level of individual variation inclusion when estimating AAD, which will lead to an increase in accuracy levels.

5. Conclusions

The initial aim of the present dissertation was to test the reliability of different AAD estimation methods based on BD. The results revealed a tendency for an underestimation of AAD, suggesting the existence of different developmental rates between the HRTS sample and the samples used in the BD methods applied.

Based on the results obtained, the methods with a more reliable AAD estimate had samples from different geographical origins, which may indicate that geographical origin is not a determinant factor for AAD estimation, as previously thought. These methods also tended to have larger samples, with the most common SES descriptions being "Low" and "Low to middle".

In individuals without epiphyseal union, the methods with the most reliable results used dry bone samples as study material and focused on measurements of the long bones from the upper and lower limb, as well as the development and epiphyseal union of the sacrum.

In individuals with partial/complete epiphyseal union, the methods with the most reliable results were also based on dry bone samples but focused on only one skeletal element from the upper or lower limb (specifically the medial clavicle, the proximal humerus and the iliac crest). The most common methodological approaches were the 3, 5 and 8 stages of epiphyseal union, as described by Cardoso (2008a, 2008b), Kellinghaus et al. (2010) and Schmeling et al. (2004), respectively. The results suggest that, for the HRTS sample, a combination of different AAD estimation methods based on a single skeletal element (preferably combining methods based on skeletal elements from the upper and lower limbs) provide the most reliable AAD estimates.

From the results obtained, it is possible to conclude that a secular growth trend did not affect the reliability of the BD methods based on epiphyseal union. More than half of the BD methods that gave a coincident AAD estimate to the reference methods had samples dated from the 21st Century, even though the HRTS sample was dated from the first half of the 18th Century. This may indicate that the sample's chronology is not a determining factor for obtaining an accurate AAD estimation based on the epiphyseal union.

It is also important to notice that methods that reported a higher accuracy were not the ones with the most reliable results when estimating AAD in the HRTS samples. This may constitute another factor supporting the idea of different developmental rates between different samples.

The high degree of SIC observed in the HRTS sample and the estimated low SES based on the social and historical context of the sample serve as markers of inadequate nutrition and frailty during life. This may lead to different developmental rates between the HRTS sample and the BD methods' samples.

Notwithstanding the results obtained, some of the most common difficulties reported by the BD methods' authors were the small samples available for study, the unequal sex and age distribution of the samples, influencing statistical results, and the different methodological approaches used, that impedes comparisons between methods to assess which ones are more accurate and reliable (Cardoso & Ríos, 2011; Ekizoglu et al., 2019a, 2019b; García-González et al., 2019; Gurses & Altinsoy, 2021; Kocasarac et al., 2016; Milenkovic et al., 2013; Oldrini et al., 2016; Torimitsu et al., 2019).

The results obtained also reinforce the importance of considering individual variation when providing an AAD estimation, since even small samples such as the HRTS may have outliers. Methods with more reliable results also had wider age ranges, which made the AAD estimates less precise but more accurate. In these cases, professional experience has an important role to aid in age estimation in both dead and living individuals. The Minimum Age Principle is in agreement with these conclusions, since it supports the notion that, when estimating AAD, the minimum age proposed in the age range should be the minimum age in which that developmental stage was found in the sample used to build

the BD method. In this way, the outliers have a smaller possibility of being excluded from the chronological age estimated for each developmental stage.

For further assessment of the results from the present dissertation, it would be useful to test these same hypotheses on a sample of known AAD and with different SES to determine any possible correlation to SIC presence and IND discrepancies. The use of Artificial Intelligence in AAD estimation methods has increased due to new developments in technology. It would also be useful to determine if these methods provide more reliable results when estimating AAD, or if the samples difficulties (such as the difficulties in obtaining large samples, with equal sex and age distribution) will remain the same.

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* References marked with an * indicate the BD and DD methods used in this dissertation

Appendix A: Informative Tables

Table A. 1.: *Table regarding information about the fifty BD methods included in the present dissertation, including samples' geographical origin, SES, chronology and sample size, as well as information regarding study material, anatomical area used, methodological approach as statistical results regarding methods accuracy, reliability and repeatability.*

Methods' Sample	Authors	Country	Continent	Century	N	F/M/I	Age Range	SES	Study Material	Bone	Skeleton area	Axial/Appendicular Skeleton	Methodological approach	Confidence interval	Accuracy	Inaccuracy	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Coefficient of variation	p-value
1	Coquerugue & Weaver, 2007	Portugal	Europe	19th-20th Century	137	69:68:0	7-39	Low	Dry Bone	Combined anatomical areas	Cranium Postcranial skeleton	Axial/Appendicular Skeleton	3 stages of epiphyseal union									
2	Cardoso, 2008a	Portugal	Europe	20th Century	106	57:49:0	0-25	Low to middle	Dry Bone	Combined anatomical areas	Lower Limb	Appendicular Skeleton	3 stages of epiphyseal union									
3	Cardoso, 2008b	Portugal	Europe	20th Century	121	65:56:0	0-20	Low to middle	Dry Bone	Combined anatomical areas	Upper Limb	Appendicular Skeleton	3 stages of epiphyseal union			100%						
4	Passalacqua, 2009	USA	North America	20th Century	634	246:388:0	14-96	Unknown, Not reported	Dry Bone	Sacrum	Vertebral Column	Axial Skeleton	Morphology	68%-95%	93.3%-99.1%	2.4%-12.39%						
5	White & Folkens, 2005	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Unknown, Not reported	Dry Bone	Combined anatomical areas	Cranium Postcranial skeleton	Axial/Appendicular Skeleton	3 stages of epiphyseal union									
6	Schaefer et al., 2009	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Unknown, Not reported	Dry Bone	Combined anatomical areas	Cranium Postcranial skeleton	Axial/Appendicular Skeleton	Measurements									
7	Boccone et al., 2010	Egypt	Africa	2900 - 1750 BCE	130	Not reported	0 - 18.5	Low to middle	Dry Bone	Combined anatomical areas	Upper Limb Lower Limb	Appendicular Skeleton	Measurements		98%				0.73-0.92	0.89-27.10 (mouth)	24.0-40.0 (mouth)	
8	Cardoso & Severino, 2010	Portugal	Europe	20th Century	92	49:43:0	0-22	Low to middle	Dry Bone	Combined anatomical areas	Upper Limb Lower Limb	Appendicular Skeleton	3 stages of epiphyseal union		99.5%		99.0%					
9	Passalacqua, 2011	USA	North America	20th Century	32	Not reported	1-19	Unknown, Not reported	Dry Bone	Calcaneus	Lower Limb	Appendicular Skeleton	3 stages of epiphyseal union, Measurements						0.862-0.900	0.018-1.88		0-0.175
10	Cardoso and Saer, 2011	Portugal	Europe	20th Century	104	57:47:0	0-30	Low to middle	Dry Bone	Vertebrae	Vertebral Column	Axial Skeleton	3 stages of epiphyseal union		87.0%-96.4%	70.8%-92.7%						
11	Nikolaković et al., 2013	Serbia	Europe	20th-21st Century	67	25:42:0	20-90	Middle to high	Human cadavers	Clavicle	Upper Limb	Appendicular Skeleton	Morphology	95%				0.851				
12	Cardoso et al., 2013a	Portugal	Europe	20th Century	148	69:79:0	0 - 20	Low to middle	Dry Bone	Combined anatomical areas	Lower Limb	Appendicular Skeleton	6 stages of epiphyseal union			100%	90%-100%					
13	Rösseck et al., 2013	Spain Portugal Bolivia	Europe	19th-20th Century	346	173:173:0	0-97	Low to middle	Dry Bone	Combined anatomical areas	Upper Limb Lower Limb	Appendicular Skeleton	Measurements					0.840-0.970	0.73-2.09			
14	Cardoso et al., 2013b	Portugal	Europe	20th Century	64	29:35:0	0-8	Low to middle	Dry Bone	Occipital	Cranium	Axial Skeleton	3 stages of epiphyseal union			99%-100%	99%-100%					
15	Hillewig et al., 2013	Germany	Europe	21st Century	220	108:108:0	16-26	Middle to high	MRI	Clavicle	Upper Limb	Appendicular Skeleton	4 stages of epiphyseal union	95%								

Table A.1. *(Continuation)*

Methods' Sample	Authors	Country	Continent	Century	N	F/M/I	Age Range	SES	Study Material	Bone	Skeleton area	Axial/Appendicular Skeleton	Methodological approach	Confidence Interval	Accuracy	Sensitivity	Specificity	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Correlation coefficient	p-value
16	Wittschieber et al., 2013	Germany	Europe	21st Century	566	253/313/0	out/30	Unknown/Not reported	X-ray	Ilium	Lower Limb	Appendicular Skeleton	8 stages of epiphyseal union										
17	Cardoso et al., 2014	Portugal	Europe	20th Century	191	90/101/0	0 - 30	Low to middle	Dry Bone	Sacrum	Vertebral Column	Axial Skeleton	4 stages of epiphyseal union					100%	87%-96%				
18	Kramer et al., 2014	Germany	Europe	21st Century	290	124/166/0	out/30	Unknown/Not reported	MRI	Tibia	Lower Limb	Appendicular Skeleton	8 stages of epiphyseal union										
19	Schaefer et al., 2015	USA	North America	Not reported	453	189/264/0	out/21	Low to high	X-ray	Combined anatomical areas	Upper Limb	Appendicular Skeleton	1-4 stages of epiphyseal union	95%									
20	Ekizoglu et al., 2015	Turkey	Asia	21st Century	503	141/362/0	out/35	Low	CT scan	Clavicle	Upper Limb	Appendicular Skeleton	5 stages of epiphyseal union	95%				82.6%	86.9%	0.734-0.741	0.145-0.868		
21	Zhang et al., 2015	China	Asia	21st Century	1777	717/1060/0	14 - 26	Low to high	X-ray	Ilium	Lower Limb	Appendicular Skeleton	8 stages of epiphyseal union	95%				0.912					
22	Dudzik and Langley, 2015	USA	North America	20th Century	248	83/148/0	18-40	Unknown/Not reported	Dry Bone	Pubis	Lower Limb	Appendicular Skeleton	Morphology	95%	70%-100%						0.832-1.000		
23	Primeau et al., 2016	Denmark	Europe	11th-16th Century	183	0/0/183	0-early adulthood	Low	X-ray	Combined anatomical areas	Upper Limb/Lower Limb	Appendicular Skeleton	Measurements							0.890-0.942	1.31-1.95		
24	Cardoso et al., 2016	Portugal	Europe	18th-20th Century	148	56/92/0	0-12	Low to high	Dry Bone	Combined anatomical areas	Upper Limb/Lower Limb	Appendicular Skeleton	Measurements	95%				>98.91%	>99.28%	0.30-0.94	0.20-3.07		
25	Al-Qtaitat et al., 2016	Jordan	Asia	21st Century	101	45/56/0	dez/22	Middle	X-ray	Combined anatomical areas	Upper Limb	Appendicular Skeleton	5 stages of epiphyseal union										
26	Galic et al., 2016	Italy	Europe	21st Century	446	212/234/0	dez/26	Unknown/Not reported	X-ray	Combined anatomical areas	Lower Limb	Appendicular Skeleton	3 stages of epiphyseal union	95%	0.90-0.95	0.89-0.94	0.92-0.96						
27	Kocasarac et al., 2016	Turkey	Asia	21st Century	116	73/43/0	ago/28	Unknown/Not reported	CT scan	Combined anatomical areas	Cranium/Dentition/ Vertebral Column	Axial Skeleton	Morphology	95%								r=0.449-0.851	<0.001
28	Oldrini et al., 2016	France	Europe	21st Century	456	148/308/13	13-96	Unknown/Not reported	CT scan	Sternum	Rib Cage	Axial Skeleton	Morphology	95%	13%-88%							0.6-0.65	
29	Sullivan et al., 2017	Australia	Oceania	21st Century	562	270/292/0	0- 30	Unknown/Not reported	CT scan	Combined anatomical areas	Lower Limb	Appendicular Skeleton	3 stages of epiphyseal union	95%							3.59-3.73		
30	Pérez et al., 2017	Spain	Europe	19th-21st Century	108	45/63/0	5 m. gest. - 6y	Unknown/Not reported	Dry Bone	Ilium	Lower Limb	Appendicular Skeleton	Measurements	95%						0.89-0.93			

Table A.1. *(Continuation)*

Methods' Sample	Authors	Country	Continent	Century	N	F/M/I	Age Range	SES	Study Material	Bone	Skeleton area	Axial/Appendicular Skeleton	Methodological approach	Confidence Interval	Accuracy	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean
31	Nougarolis et al., 2017	France	Europe	21st Century	232	109/123/0	8-30	Unknown/Not reported	CT scan	Scapula	Upper Limb	Appendicular Skeleton	5 stages of epiphyseal union						
32	Olivares and Aguilera, 2017	Spain	Europe	20th Century	114	43/71/0	5 m. gest. - 6 y.	Unknown/Not reported	Dry Bone	Occipital	Cranium	Axial Skeleton	Measurements	95%				0.68-0.9	
33	Lottering et al., 2017	Australia	Oceania	21st Century	524	241/283/0	6-23	Unknown/Not reported	CT scan	Ilium	Lower Limb	Appendicular Skeleton	7 stages of epiphyseal union	95%					
34	Alhazmi et al., 2017	USA	North America	21st Century	741	380/361/0	6-20	Unknown/Not reported	CT scan	Occipital	Cranium	Axial Skeleton	4 stages of epiphyseal union	95%		93%-94%	91%	0.667-0.680	1.618-1.624
35	Pate et al., 2018	India	Asia	Not reported	198	81/117/0	8-26	Unknown/Not reported	Human cadavers	Occipital	Cranium	Axial Skeleton	3 stages of epiphyseal union	95%				0.6704/0.7636	
36	Marera & Satyapal, 2018	South Africa/Kenya	Africa	Not reported	1605	800/805/0	14 - 30	Low to middle	X-ray	Clavicle	Upper Limb	Appendicular Skeleton	5 stages of epiphyseal union	95%					1.43/1.44
37	Mahon et al., 2018	South Africa	Africa	20th Century	211	110/101/0	12-45	Unknown/Not reported	Dry Bone	Clavicle	Upper Limb	Appendicular Skeleton	3 stages of epiphyseal union						
38	Campanacho et al., 2018	Portugal	Europe	Not reported	68	35/33/0	1-30	Unknown/Not reported	Dry Bone	Sternum	Rib Cage	Axial Skeleton	3 stages of epiphyseal union						
39	Koterová et al., 2018	Portugal/Switzerland /Spain/USA/South Africa/Thailand	Various	19th-20th Century	941	472/469/0	19-100	Low to middle	Dry Bone	Combined anatomical areas	Lower Limb	Appendicular Skeleton	Morphology		30.7%-72.3%				9.7-11.0
40	Chowdhuri er al., 2019	India	Asia	21st Century	157	65/92/0	10-25	Unknown/Not reported	X-ray	Ilium	Lower Limb	Appendicular Skeleton	8 stages of epiphyseal union						0.259-0.298
41	Ekizoglu et al., 2019a	Turkey	Asia	21st Century	428	188/240/0	12-31	Low	MRI	Humerus	Upper Limb	Appendicular Skeleton	5 stages of epiphyseal union						
42	Garcia-Gonzalez et al., 2019	Portugal	Europe	19th-20th Century	177	89/88/0	0 - 26	Low to middle	Dry Bone	Femur	Lower Limb	Appendicular Skeleton	5 stages of epiphyseal union						
43	Ekizoglu et al., 2019b	Turkey	Asia	21st Century	395	173/222/0	12-30	Low	MRI	Humerus	Upper Limb	Appendicular Skeleton	5 stages of epiphyseal union						
44	Corron et al., 2019	France	Europe	21st Century	594	?/?/?	0-19	Unknown/Not reported	CT scan	Combined anatomical areas	Upper Limb/Lower Limb/Vertebral Column	Axial/Appendicular Skeleton	Measurements	95%	>95%				0.257-37.54
45	El-Din et al., 2019	Egypt	Africa	21st Century	335	118/217/0	8-28	Unknown/Not reported	MRI	Tibia	Lower Limb	Appendicular Skeleton	9 stages of epiphyseal union						

Table A.1. *(Continuation)*

Methods' Sample	Authors	Country	Continent	Century	N	F/M/I	Age Range	SES	Study Material	Bone	Skeleton area	Axial/Appendicular Skeleton	Methodological approach	Confidence Interval	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean
46	Torimitsu et al., 2019	Japan	Asia	21st Century	207	79/128/0	12-30	Unknown/Not reported	CT scan	Clavicle	Upper Limb	Appendicular Skeleton	9 stages of epiphyseal union					
47	Uys et al., 2019	South Africa	Africa	21st Century	974	513/461/0	15-22	Low to high	X-ray	Vertebrae	Vertebral Column	Axial Skeleton	5 stages of epiphyseal union	95%		58%-70%	0.49-0.71	1.19-1.65
48	Gurses and Altinsoy, 2020	Turkey	Asia	21st Century	598	231/367/0	12-30	Unknown/Not reported	MRI	Combined anatomical areas	Lower Limb	Appendicular Skeleton	5 stages of epiphyseal union	95%	94%	97.8%-98.5%		
49	Hocaoglu et al., 2020	Turkey	Asia	21st Century	1276	471/805/0	10-25	Unknown/Not reported	CT scan	Vertebrae	Vertebral Column	Axial Skeleton	5 stages of epiphyseal union	95%				
50	Singh et al., 2020	India	Asia	21st Century	102	102/0/0	13-20	Low	X-ray	Combined anatomical areas	Lower Limb	Appendicular Skeleton	3 stages of epiphyseal union					1-2 years

Table A. 2.: *Table regarding information about the six DD methods included in the present dissertation, including samples' geographical origin, SES, chronology, and sample size, as well as information regarding study material, anatomical area used, methodological approach as statistical results regarding methods accuracy, reliability, and repeatability.*

Methods' Sample	Authors	Country	Continent	Century	N	F/M/I	Age Range	SES	Study Material	Bone	Skeleton area	Axial/Appendicular Skeleton	Methodological approach	Intra-observer agreement	Inter-observer agreement	R2	p-value
51	Moorrees et al., 1963a	USA	North America	20th Century	345	161/184/0	0-25	Middle	X-ray	Permanent Dentition	Dentition	Axial Skeleton	Morphology/Development				
52	Moorrees et al., 1963b	USA	North America	20th Century	246	110/136/0	0-15	Middle	X-ray	Deciduous Dentition	Dentition	Axial Skeleton	Morphology/Development				
53	Ubelaker, 1989	USA	North America	20th Century				Unknown/Not reported	X-ray	Permanent and Deciduous Dentition	Dentition	Axial Skeleton	Morphology/Development				
54	AlQahtani et al., 2010	ritain	Europe	18th-21st Century	704	336/355/13	7 m. gest. - 24y.	Low to middle	Dry Bone/X-ray	Permanent and Deciduous Dentition	Dentition	Axial Skeleton	Morphology/Development				
55	Irurita et al., 2014	Spain	Europe	19th-21st Century	138	58/80/0	6 m. gest. - 5 y.	Unknown/Not reported	Dry Bone	Deciduous Dentition	Dentition	Axial Skeleton	Morphology/Development	76%-94.2%	81.7%-89.1%		
56	Arthanari et al., 2020	India	Asia	21st Century	200	100/100/0	10-19.9	Unknown/Not reported	X-ray	Permanent Second and Third Molars	Dentition	Axial Skeleton	Morphology/Development			0.353-0.829	0.000-0.013

Table A. 3.: *Informative table about the BD methods from Group_1: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence Interval	Accuracy	Intra-observer agreement	Inter-observer agreement	Correlation coefficient
1	Coqueugniot & Weaver, 2007					
5	White & Folkens, 2009					
16	Wittschieber et al., 2013			0.817-0.899	0.753-0.822	
28	Oldrini et al., 2016	95%	13%-88%		0.86	0.6-0.65
42	García-Gonzalez et al., 2019			0.98	0.96	

Table A. 4.: *Informative table relating to the AAD estimates obtained from Group_1's BD methods: the minimum and maximum age ranges provided by each method, as well as the individuals in which this estimate was obtained, and the average interval (in years) for each method.*

Methods' Number	Min. Age Range	Min. Interval (Years)	Individual(s)	Max. Age Range	Max. Interval (Years)	Individual(s)	Average (Years)
1	22-23	2	[573]	20-29	10	[1314]	5,4
5	22	1	[1296]; [1313]	22-30	9	[573]	5,3
16	12-17; 16-21; 15-20	6	[575]; [1214]; [1419]	16-30	15	[573]; [1406]; [1429]	18,9
28	25	1	[1406]; [1429]	0-20	21	[575]	6,6
42	11-17	7	[575]	16-35	20	[1429]	14,1

Table A. 5.: *Informative table about the BD methods from Group_2: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence Interval	Accuracy	Sensitivity	Specificity	Intra-observer agreement	Inter-observer agreement
2	Cardoso, 2008a						
8	Cardoso & Severino, 2010					99.5%	99.0%
11	Milenkovic et al., 2013	95%					0.851
17	Cardoso et al., 2014					100%	87%-96%
18	Kramer et al., 2014					0.88	0.85
26	Galic et al., 2016	95%	0.90-0.95	0.89-0.94	0.92-0.96	0.839-0.907	0.791-0.19
31	Nougarolis et al., 2017					0.85-1	0.72-1
33	Lottering et al., 2017	95%				ICC=0.933	ICC=0.863
41	Ekizoglu et al., 2019a					0.898	0.828
46	Torimitsu et al., 2019					0.907	0.808
48	Gurses and Altinsoy, 2020	95%				94%	97.8%-98.5%

Table A. 6.: *Informative table relating to the AAD estimates obtained from Group_2's BD methods: the minimum and maximum age ranges provided by each method, as well as the individuals in which this estimate was obtained, and the average interval (in years) for each method.*

Methods' Number	Min. Age Range	Min. Interval (Years)	Individual(s)	Max. Age Range	Max. Interval (Years)	Individual(s)	Average (Years)
2	18-21	4	[1296]	18-35	18	[1429]	7,7
8	18-30	13	[1406]; [1419]	18-35	18	[1429]	14,6
11	31-35	5	[1429]	0-22	23	[575]	11,1
17	18-21	4	[573]	0-18	19	[575]	10,7
18	19-23	5	[1406]	15-31	17	[1429]	14,3
26	18-20	3	[1419]	15-36	22	[1429]	9,6
31	16-19	4	[1419]	16-30	15	[1429]	11,7
33	15-19	5	[1419]	17-35	19	[1429]	11,6
41	16-23	8	[573]; [1214]; [1296]; [1419]	17-30	14	[1406]; [1429]	10,4
46	12-17; 16-21	6	[575]; [1419]	19-30	12	[1429]	8,4
48				20-30	11	[573]; [1214]; [1296]; [1313]; [1406]; [1419]; [1429]	11

Table A. 7.: *Informative table relating to the AAD estimates obtained from Group_Dentition1's methods: the minimum and maximum age ranges provided by each method, as well as the individuals in which this estimate was obtained, and the average interval (in years) for each method*

Methods' Number	Min. Age Range	Min. Interval (Years)	Individual(s)	Max. Age Range	Max. Interval (Years)	Individual(s)	Average (Years)
20	10-17	8	[575]	16-29	14	[1296], [1406]	11.5
21	14-19	6	[575]	16-25	10	[1296]	8.3
36	26-30	5	[1429]	16-26	11	[1296], [1406]	8.7
37	20-30	11	[1296], [1406]	13-30	18	[1214]	14
40	17-22	6	[1214]	12-25	14	[1296]	9.3
43	12-17	6	[575]	16-30	15	[1214]	11

Table A. 8.: *Informative table about the BD methods from Group_Dentition1: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Author(s), Year	Confidence interval	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean
20	Ekizoglu et al., 2015	95%	82.6%	86.9%	0.734-0.741	0.145-0.868
21	Zhang et al., 2015	95%	0.912			
36	Marera & Satyapal, 2018	95%				1.43/1.44
37	Mahon et al., 2018		0.91	0.78		
40	Chowdhuri et al., 2019		0.61-0.80	0.61-0.80		0.259-0.298
43	Ekizoglu et al., 2019b		0.818	0.798		

Table A. 9.: *Informative table about the BD methods from Group_Dentition2: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence interval	Accuracy	Intra-observer agreement	Inter-observer agreement	Correlation coefficient
1	Coqueugniot & Weaver, 2007					
2	Cardoso, 2008a					
5	White & Folkens, 2009					
8	Cardoso & Severino, 2010			99.5%	99.0%	
10	Cardoso and Ríos, 2011			87.0%-95.4%	79.8%-92.7%	
11	Milenkovic et al., 2013	95%			0.851	
16	Wittschieber et al., 2013			0.817-0.899	0.753-0.822	
28	Oldrini et al., 2016	95%	13%-88%		0.86	0.6-0.65
31	Nougarolis et al., 2017			0.85-1	0.72-1	

Table A. 10.: *Informative table relating to the AAD estimates obtained from Group_Dentition2's methods: the minimum and maximum age ranges provided by each method, as well as the individuals in which this estimate was obtained, and the average interval (in years) for each method.*

Methods' Number	Min. Age Range	Min. Interval (Years)	Individual(s)	Max. Age Range	Max. Interval (Years)	Individual(s)	Average (Years)
1	20-22; 25-27	3	[1296]; [1429]	20-29	10	[1314]	5.8
2	18-21	4	[1296]	18-35	18	[1429]	8.8
5	22	1	[1296]	11-18; 23-30	8	[575]; [1314]	5.8
8	18-30	13	[1406]	18-35	18	[1429]	14.8
10	17-23	7	[1214]; [1296]	18-35	18	[1429]	12
11	31-35	5	[1429]	0-22	23	[1214]	11.6
16	12-17;16-21	6	[575]; [1214]	16-30	15	[1406]; [1429]	11.2
28	25	1	[1406]; [1429]	0-20	21	[575]	7
31	15-25	11	[1214]	16-30	15	[1429]	13

Table A. 11.: *Informative table about the BD methods from Group_No_Dentition: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence interval	Accuracy	Sensitivity	Specificity	Intra-observer agreement	Inter-observer agreement	Standar error of mean	Correlation coefficient
1	Coqueugniot & Weaver, 2007								
5	White & Folkens, 2009								
10	Cardoso and Ríos, 2011					87.0%-95.4%	79.8%-92.7%		
16	Wittschieber et al., 2013					0.817-0.899	0.753-0.822		
17	Cardoso et al., 2014					100%	87%-96%		
18	Kramer et al., 2014					0.88	0.85		
26	Galic et al., 2016	95%	0.90-0.95	0.89-0.94	0.92-0.96	0.839-0.907	0.791-0.19		
28	Oldrini et al., 2016	95%	13%-88%				0.86		0.6-0.65
29	Sullivan et al., 2017	95%				0.75-0.88		3.59-3.73	
33	Lottering et al., 2017	95%				ICC=0.933	ICC=0.863		
42	García-Gonzalez et al., 2019					0.98	0.96		
45	El-Din et al., 2019					0.92	0.85		
48	Gurses and Altinsoy, 2020	95%				94%	97.8%-98.5%		

Table A. 12.: *Informative table relating to the AAD estimates obtained from Group_No_Dentition's methods: the minimum and maximum age ranges provided by each method, as well as the individuals in which this estimate was obtained, and the average interval (in years) for each method*

Methods' Number	Min. Age Range	Min. Interval (Years)	Individual(s)	Max. Age Range	Max. Interval (Years)	Individual(s)	Average (Years)
1	22-23	2	[573]	19-26	8	[1313]	4.7
5	22	1	[1313]	22-30	9	[573]	4.3
10	18-23	6	[1419]	17-30	14	[1313]	11
16	15-20	6	[1419]	16-30	15	[573]	10.3
17	18-21	4	[573]	16-30	15	[1419]	8
18	16-30	15	[1419]	15-30	16	[573]; [1313]	15.7
26	18-20	3	[1419]	15-24	10	[573]	6.3
28				20-25	6	[573]; [1313]; [1419]	6
29				17-24; 13-20	8	[573]; [1313]; [1419]	8
33	15-19	5	[1419]	17-30	14	[573]	8.7
42	18-30	13	[1419]	16-30	15	[573]; [1313]	14.3
45	19-24	6	[1419]	21-27; 18-24	7	[573]; [1313]	6.7
48				20-30	11	[573]; [1313]; [1419]	11

Table A. 13.: *Informative table about the BD methods from Group_Partial_Fusion: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence interval	Accuracy	Intra-observer agreement	Inter-observer agreement	Correlation coefficient
10	Cardoso and Ríos, 2011			87.0%-95.4%	79.8%-92.7%	
1	Coqueugniot & Weaver, 2007					
2	Cardoso, 2008a					
5	White & Folkens, 2009					
8	Cardoso & Severino, 2010			99.5%	99.0%	
11	Milenkovic et al., 2013	95%			0.851	
16	Wittschieber et al., 2013			0.817-0.899	0.753-0.822	
18	Kramer et al., 2014			0.88	0.85	
28	Oldrini et al., 2016	95%	13%-88%		0.86	0.6-0.65
31	Nougarolis et al., 2017			0.85-1	0.72-1	
41	Ekizoglu et al., 2019a			0.898	0.828	
42	García-Gonzalez et al., 2019			0.98	0.96	
48	Gurses and Altinsoy, 2020	95%		94%	97.8%-98.5%	

Table A. 14.: *Informative table relating to the AAD estimates obtained from Group_Partial_Fusion's methods: the minimum and maximum age ranges provided by each method, as well as the individuals in which this estimate was obtained, and the average interval (in years) for each method.*

Methods' Number	Min. Age Range	Min. Interval (Years)	Individual(s)	Max. Age Range	Max. Interval (Years)	Individual(s)	Average (Years)
10	18-23	6	[1419]	18-35	18	[1429]	11.6
1	22-23	2	[573]	20-29	10	[1314]	5.4
2	18-21	4	[1296]	18-35	18	[1429]	7.7
5	22	1	[1296]; [1313]	22-30	9	[573]	4.9
8	18-30	13	[1406]; [1419]	18-35	18	[1429]	14.6
11	31-35	5	[1429]	0-22	23	[1214]	11.1
16	16-21; 15-20	6	[1214]; [1419]	16-30	15	[1314]; [1406]; [1429]	11.6
18	19-23	5	[1406]	15-31	17	[1429]	14.3
28	25	1	[1406]; [1429]	20-25; 25-30	6	[1296]; [1314]; [573]; [1313]; [1419]	4.6
31	16-19	4	[1419]	16-30	15	[1429]	11.7
41	16-23	8	[1214]; [1296]; [573]; [1419]	17-30	14	[1406]; [1429]	10.4
42	18-30	13	[1214]; [1419]	16-35	20	[1429]	15.1
48				20-30	11	[1214]; [1296]; [1406]; [1429]; [573]; [1313]; [1419]	11

Table A. 15.: *Informative table about the BD methods from Group_No_Fusion: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean
7	Boccone et al., 2010	98%		0.73-0.92	0.89-27.10 (month)
12	Cardoso et al., 2013a	100%	90%-100%		
13	Rissech et al., 2013			0.840-0.970	0.73-2.09
17	Cardoso et al., 2014	100%	87%-96%		
23	Primeau et al., 2016		0.890-0.942	1.31-1.95	

Table A. 16.: *Informative table relating to the AAD estimates obtained from Group_No_Fusion's methods: the minimum and maximum age ranges provided by each method, as well as the individuals in which this estimate was obtained, and the average interval (in years) for each method.*

Methods' Number	Min. Age Range	Min. Interval (Years)	Individual(s)	Max. Age Range	Max. Interval (Years)	Individual(s)	Average (Years)
7	14.5-15.5	2	[575]	2.5-5.5	4	[1008]	3
12				0-9	10	[872]; [1008]	10
13	15-16.5	2	[575]	0-2.5	3	[872]	2.5
17	0-4	5	[872]	0-18	19	[575]	12
23	3-6	4	[1008]	0-4	5	[872]	4.5

Table A. 17.: *Informative table about the BD methods from Group_573: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Author(s), Year	Confidence Interval	Accuracy	Sensitivity	Specificity	Innacuracy	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Correlation coefficient	p-value
1	Coqueugniot & Weaver, 2007											
2	Cardoso, 2008a											
4	Passalacqua, 2009	68%-95%	93.3%-99.1%			2.6%-12.39%						
5	White & Folkens, 2009											
8	Cardoso & Severino, 2010						99.5%	99.0%				
10	Cardoso and Rios, 2011						87.0%-95.4%	79.8%-92.7%				
11	Milenkovic et al., 2013	95%						0.851				
15	Hillewig et al., 2013	95%					0.75	0.74-0.76				
16	Wittschieber et al., 2013						0.817-0.899	0.753-0.822				
17	Cardoso et al., 2014						100%	87%-96%				
18	Kramer et al., 2014						0.88	0.85				
26	Galic et al., 2016	95%	0.90-0.95	0.89-0.94	0.92-0.96		0.839-0.907	0.791-0.19				
27	Kocasarac et al., 2016	95%					0.841-0.889				r=0.449 - 0.851	<0.001
28	Oldrini et al., 2016	95%	13%-88%					0.86			0.6-0.65	
29	Sullivan et al., 2017	95%					0.75-0.88			3.59-3.73		
31	Nougarolis et al., 2017						0.85-1	0.72-1				
33	Lottering et al., 2017	95%					ICC=0.933	ICC=0.863				
34	Alhazmi et al., 2017	95%					93%-94%	91%	0.667-0.680	1.618-1.624		
35	Pate et al., 2018	95%							0.6704/0.7636			
41	Ekizoglu et al., 2019a						0.898	0.828				
42	Garcia-Gonzalez et al., 2019						0.98	0.96				
45	El-Din et al., 2019						0.92	0.85				
46	Torimitsu et al., 2019						0.907	0.808				
47	Uys et al., 2019	95%						58%-70%	0.49-0.71	1.19-1.65		
48	Gurses and Altinsoy, 2020	95%					94%	97.8%-98.5%				
49	Hocaoglu et al., 2020	95%					0.972-0.981	0.934-0.972				
50	Singh et al., 2020									1-2 years		

Table A. 18.: *Informative table about the BD methods from Group_575: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence interval	Accuracy	Sensitivity	Specificity	Innacuracy	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Correlation coefficient	Coefficient of variation
1	Coqueugniot & Weaver, 2007											
4	Passalacqua, 2009	68%-95%	93.3%-99.1%			2.6%-12.39%						
5	White & Folkens, 2009											
7	Boccone et al., 2010						98%		0.73-0.92	0.89-27.10 (month)		24.0-40.0 (month)
13	Rissech et al., 2013								0.840-0.970	0.73-2.09		
16	Wittschieber et al., 2013						0.817-0.899	0.753-0.822				
17	Cardoso et al., 2014						100%	87%-96%				
19	Schaefer et al., 2015	95%					0.86-0.95	0.80-0.851				
20	Ekizoglu et al., 2015	95%					82.6%	86.9%	0.734-0.741	0.145-0.868		
21	Zhang et al., 2015	95%					0.912					
26	Galic et al., 2016	95%	0.90-0.95	0.89-0.94	0.92-0.96		0.839-0.907	0.791-0.19				
28	Oldrini et al., 2016	95%	13%-88%					0.86			0.6-0.65	
33	Lottering et al., 2017	95%					ICC=0.933	ICC=0.863				
36	Marera & Satyapal, 2018	95%								1.43/1.44		
37	Mahon et al., 2018						0.91	0.78				
40	Chowdhuri er al., 2019						0.61-0.80	0.61-0.80		0.259-0.298		
42	García-Gonzalez et al., 2019						0.98	0.96				
43	Ekizoglu et al., 2019b						0.818	0.798				
46	Torimitsu et al., 2019						0.907	0.808				
47	Uys et al., 2019	95%						58%-70%	0.49-0.71	1.19-1.65		

Table A. 19.: *Informative table about the BD methods from Group_872: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence Interval	Accuracy	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean
6	Schaefer et al., 2009						
12	Cardoso et al., 2013a			100%	90%-100%		
13	Rissech et al., 2013					0.840-0.970	0.73-2.09
14	Cardoso et al., 2013b			90%-100%	90%-100%		
17	Cardoso et al., 2014			100%	87%-96%		
23	Primeau et al., 2016				0.890-0.942	1.31-1.95	
30	Pérez et al., 2017	95%		p=0.2	p=0.2	0.89-0.93	
44	Corron et al., 2019	95%	>95%				0.257-37.54

Table A. 20.: *Informative table about the BD methods from Group_1008: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Author(s), Year	Confidence interval	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Coefficient of variation
7	Boccone et al., 2010		98%		0.73-0.92	0.89-27.10 (month)	24.0-40.0 (month)
12	Cardoso et al., 2013a		100%	90%-100%			
23	Primeau et al., 2016			0.890-0.942	1.31-1.95		
29	Sullivan et al., 2017	95%	0.75-0.88			3.59-3.73	
38	Campanacho et al., 2018						

Table A. 21.: *Informative table about the BD methods from Group_1214: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' number	Author(s), Year	Confidence interval	Accuracy	Innacuracy	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Correlation coefficient	p-value
2	Cardoso, 2008a									
3	Cardoso, 2008b				100%					
4	Passalacqua, 2009	68%-95%	93.3%-99.1%	2.6%-12.39%						
8	Cardoso & Severino, 2010				99.5%	99.0%				
10	Cardoso and Ríos, 2011				87.0%-95.4%	79.8%-92.7%				
11	Milenkovic et al., 2013	95%				0.851				
16	Wittschieber et al., 2013				0.817-0.899	0.753-0.822				
17	Cardoso et al., 2014				100%	87%-96%				
18	Kramer et al., 2014				0.88	0.85				
20	Ekizoglu et al., 2015	95%			82.6%	86.9%	0.734-0.741	0.145-0.868		
21	Zhang et al., 2015	95%			0.912					
25	Al-Qtaitat et al., 2016									
27	Kocasarac et al., 2016	95%			0.841-0.889				r=0.449-0.851	<0.001
31	Nougarolis et al., 2017				0.85-1	0.72-1				
34	Alhazmi et al., 2017	95%			93%-94%	91%	0.667-0.680	1.618-1.624		
35	Pate et al., 2018	95%					0.6704/0.7636			
36	Marera & Satyapal, 2018	95%						1.43/1.44		
37	Mahon et al., 2018				0.91	0.78				
40	Chowdhuri er al., 2019				0.61-0.80	0.61-0.80		0.259-0.298		
41	Ekizoglu et al., 2019a				0.898	0.828				
42	García-Gonzalez et al., 2019				0.98	0.96				
43	Ekizoglu et al., 2019b				0.818	0.798				
45	El-Din et al., 2019				0.92	0.85				
48	Gurses and Altinsoy, 2020	95%			94%	97.8%-98.5%				

Table A. 22.: *Informative table about the BD methods from Group_1296: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence interval	Accuracy	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Correlation coefficient	p-value
1	Coqueugniot & Weaver, 2007								
2	Cardoso, 2008a								
5	White & Folkens, 2009								
8	Cardoso & Severino, 2010			99.5%	99.0%				
10	Cardoso and Ríos, 2011			87.0%-95.4%	79.8%-92.7%				
11	Milenkovic et al., 2013	95%			0.851				
15	Hillewig et al., 2013	95%		0.75	0.74-0.76				
18	Kramer et al., 2014			0.88	0.85				
20	Ekizoglu et al., 2015	95%		82.6%	86.9%	0.734-0.741	0.145-0.868		
21	Zhang et al., 2015	95%		0.912					
27	Kocasarac et al., 2016	95%		0.841-0.889				r=0.449-0.851	<0.001
28	Oldrini et al., 2016	95%	13%-88%		0.86			0.6-0.65	
31	Nougarolis et al., 2017			0.85-1	0.72-1				
36	Marera & Satyapal, 2018	95%					1.43/1.44		
37	Mahon et al., 2018			0.91	0.78				
40	Chowdhuri er al., 2019			0.61-0.80	0.61-0.80		0.259-0.298		
41	Ekizoglu et al., 2019a			0.898	0.828				
42	García-Gonzalez et al., 2019			0.98	0.96				
43	Ekizoglu et al., 2019b			0.818	0.798				
46	Torimitsu et al., 2019			0.907	0.808				
48	Gurses and Altinsoy, 2020	95%		94%	97.8%-98.5%				

Table A. 23.: *Informative table about the BD methods from Group_1313: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence interval	Accuracy	Sensitivity	Specificity	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Correlation coefficient	Coefficient of variation
1	Coqueugniot & Weaver, 2007										
2	Cardoso, 2008a										
5	White & Folkens, 2009										
7	Boccone et al., 2010					98%		0.73-0.92	0.89-27.10 (month)		24.0-40.0 (month)
10	Cardoso and Ríos, 2011					87.0%-95.4%	79.8%-92.7%				
16	Wittschieber et al., 2013					0.817-0.899	0.753-0.822				
18	Kramer et al., 2014					0.88	0.85				
26	Galic et al., 2016	95%	0.90-0.95	0.89-0.94	0.92-0.96	0.839-0.907	0.791-0.19				
28	Oldrini et al., 2016	95%	13%-88%				0.86			0.6-0.65	
29	Sullivan et al., 2017	95%				0.75-0.88			3.59-3.73		
33	Lottering et al., 2017	95%				ICC=0.933	ICC=0.863				
38	Campanacho et al., 2018										
42	García-Gonzalez et al., 2019					0.98	0.96				
45	El-Din et al., 2019					0.92	0.85				
48	Gurses and Altinsoy, 2020	95%				94%	97.8%-98.5%				

Table A. 24.: *Informative table about the BD methods from Group_1314: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence interval	Accuracy	Sensitivity	Specificity	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Correlation coefficient
1	Coqueugniot & Weaver, 2007									
2	Cardoso, 2008a									
3	Cardoso, 2008b					100%				
5	White & Folkens, 2009									
8	Cardoso & Severino, 2010					99.5%	99.0%			
10	Cardoso and Ríos, 2011					87.0%-95.4%	79.8%-92.7%			
11	Milenkovic et al., 2013	95%					0.851			
15	Hillewig et al., 2013	95%				0.75	0.74-0.76			
16	Wittschieber et al., 2013					0.817-0.899	0.753-0.822			
20	Ekizoglu et al., 2015	95%				82.6%	86.9%	0.734-0.741	0.145-0.868	
21	Zhang et al., 2015	95%				0.912				
26	Galic et al., 2016	95%	0.90-0.95	0.89-0.94	0.92-0.96	0.839-0.907	0.791-0.19			
28	Oldrini et al., 2016	95%	13%-88%				0.86			0.6-0.65
31	Nougarolis et al., 2017					0.85-1	0.72-1			
33	Lottering et al., 2017	95%				ICC=0.933	ICC=0.863			
36	Marera & Satyapal, 2018	95%							1.43/1.44	
37	Mahon et al., 2018					0.91	0.78			
40	Chowdhuri er al., 2019					0.61-0.80	0.61-0.80		0.259-0.298	
41	Ekizoglu et al., 2019a					0.898	0.828			
43	Ekizoglu et al., 2019b					0.818	0.798			
46	Torimitsu et al., 2019					0.907	0.808			

Table A. 25.: *Informative table about the BD methods from Group_1406: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence interval	Accuracy	Sensitivity	Specificity	Innacuracy	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Correlation coefficient	p-value
1	Coqueugniot & Weaver, 2007											
2	Cardoso, 2008a											
3	Cardoso, 2008b						100%					
4	Passalacqua, 2009	68%-95%	93.3%-99.1%			2.6%-12.39%						
5	White & Folkens, 2009											
8	Cardoso & Severino, 2010						99.5%	99.0%				
10	Cardoso and Ríos, 2011						87.0%-95.4%	79.8%-92.7%				
11	Milenkovic et al., 2013	95%						0.851				
15	Hillewig et al., 2013	95%					0.75	0.74-0.76				
16	Wittschieber et al., 2013						0.817-0.899	0.753-0.822				
17	Cardoso et al., 2014						100%	87%-96%				
18	Kramer et al., 2014						0.88	0.85				
20	Ekizoglu et al., 2015	95%					82.6%	86.9%	0.734-0.741	0.145-0.868		
21	Zhang et al., 2015	95%					R=0.912					
22	Dudzik and Langley, 2015	95%	70%-100%					0.546-0.944		0.832-1.000		
26	Galic et al., 2016	95%	0.90-0.95	0.89-0.94	0.92-0.96		0.839-0.907	0.791-0.19				
27	Kocasarac et al., 2016	95%					0.841-0.889				r=0.449-0.851	<0.001
28	Oldrini et al., 2016	95%	13%-88%					0.86			0.6-0.65	
31	Nougarolis et al., 2017						0.85-1	0.72-1				
33	Lottering et al., 2017	95%					ICC=0.933	ICC=0.863				
34	Alhazmi et al., 2017	95%					93%-94%	91%	0.667-0.680	1.618-1.624		
36	Marera & Satyapal, 2018	95%								1.43/1.44		
37	Mahon et al., 2018						0.91	0.78				
39	Koterová et al., 2018		30.7%-72.3%							9.7-11.0		
40	Chowdhuri er al., 2019						0.61-0.80	0.61-0.80		0.259-0.298		
41	Ekizoglu et al., 2019a						0.898	0.828				
42	García-Gonzalez et al., 2019						0.98	0.96				
43	Ekizoglu et al., 2019b						0.818	0.798				
46	Torimitsu et al., 2019						0.907	0.808				
47	Uys et al., 2019	95%						58%-70%	0.49-0.71	1.19-1.65		
48	Gurses and Altinsoy, 2020	95%					94%	97.8%-98.5%				
49	Hocaoglu et al., 2020	95%					0.972-0.981	0.934-0.972				

Table A. 26.: *Informative table about the BD methods from Group_1419: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence Interval	Accuracy	Sensitivity	Specificity	Intra-observer agreement	Inter-observer agreement	Standar error of mean	Correlation coefficient
1	Coqueugniot & Weaver, 2007								
3	Cardoso, 2008b					100%			
5	White & Folkens, 2009								
8	Cardoso & Severino, 2010					99.5%	99.0%		
10	Cardoso and Ríos, 2011					87.0%-95.4%	79.8%-92.7%		
11	Milenkovic et al., 2013	95%					0.851		
15	Hillewig et al., 2013	95%				0.75	0.74-0.76		
16	Wittschieber et al., 2013					0.817-0.899	0.753-0.822		
17	Cardoso et al., 2014					100%	87%-96%		
18	Kramer et al., 2014					0.88	0.85		
19	Schaefer et al., 2015	95%				0.86-0.95	0.80-0.851		
22	Dudzik and Langley, 2015	95%	70%-100%				0.546-0.944	0.832-1.000	
25	Al-Qtaitat et al., 2016								
26	Galic et al., 2016	95%	0.90-0.95	0.89-0.94	0.92-0.96	0.839-0.907	0.791-0.19		
28	Oldrini et al., 2016	95%	13%-88%				0.86		0.6-0.65
29	Sullivan et al., 2017	95%				0.75-0.88		3.59-3.73	
31	Nougarolis et al., 2017					0.85-1	0.72-1		
33	Lottering et al., 2017	95%				ICC=0.933	ICC=0.863		
38	Campanacho et al., 2018								
41	Ekizoglu et al., 2019a					0.898	0.828		
42	García-Gonzalez et al., 2019					0.98	0.96		
45	El-Din et al., 2019					0.92	0.85		
46	Torimitsu et al., 2019					0.907	0.808		
48	Gurses and Altinsoy, 2020	95%				94%	97.8%-98.5%		

Table A. 27.: *Informative table about the BD methods from Group_1429: correspondence between methods' number and author(s) and year of publication, as well as statistical information provided by each method regarding its performance evaluation.*

Methods' Number	Authors	Confidence interval	Accuracy	Sensitivity	Specificity	Innacuracy	Intra-observer agreement	Inter-observer agreement	R2	Standar error of mean	Correlation coefficient	p-value
1	Coqueugniot & Weaver, 2007											
2	Cardoso, 2008a											
3	Cardoso, 2008b						100%					
4	Passalacqua, 2009	68%-95%	93.3%-99.1%			2.6%-12.39%						
5	White & Folkens, 2009											
8	Cardoso & Severino, 2010						99.5%	99.0%				
10	Cardoso and Rios, 2011						87.0%-95.4%	79.8%-92.7%				
11	Milenkovic et al., 2013	95%						0.851				
15	Hillewig et al., 2013	95%					0.75	0.74-0.76				
16	Wittschieber et al., 2013						0.817-0.899	0.753-0.822				
17	Cardoso et al., 2014						100%	87%-96%				
18	Kramer et al., 2014						0.88	0.85				
20	Ekizoglu et al., 2015	95%					82.6%	86.9%	0.734-0.741	0.145-0.868		
21	Zhang et al., 2015	95%					0.912					
22	Dudzik and Langley, 2015	95%	70%-100%					0.546-0.944		0.832-1.000		
26	Galic et al., 2016	95%	0.90-0.95	0.89-0.94	0.92-0.96		0.839-0.907	0.791-0.19				
27	Kocasarac et al., 2016	95%					0.841-0.889				r=0.449-0.851	<0.001
28	Oldrini et al., 2016	95%	13%-88%					0.86			0.6-0.65	
29	Sullivan et al., 2017	95%					0.75-0.88			3.59-3.73		
31	Nougarolis et al., 2017						0.85-1	0.72-1				
33	Lottering et al., 2017	95%					ICC=0.933	ICC=0.863				
34	Alhazmi et al., 2017	95%					93%-94%	91%	0.667-0.680	1.618-1.624		
36	Marera & Satyapal, 2018	95%								1.43/1.44		
37	Mahon et al., 2018						0.91	0.78				
38	Campanacho et al., 2018											
39	Koterová et al., 2018		30.7%-72.3%							9.7-11.0		
40	Chowdhuri er al., 2019						0.61-0.80	0.61-0.80		0.259-0.298		
41	Ekizoglu et al., 2019a						0.898	0.828				
42	Garcia-Gonzalez et al., 2019						0.98	0.96				
43	Ekizoglu et al., 2019b						0.818	0.798				
45	El-Din et al., 2019						0.92	0.85				
46	Torimitsu et al., 2019						0.907	0.808				
47	Uys et al., 2019	95%						58%-70%	0.49-0.71	1.19-1.65		
48	Gurses and Altinsoy, 2020	95%					94%	97.8%-98.5%				
49	Hocaoglu et al., 2020	95%					0.972-0.981	0.934-0.972				