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The Sum of One's Parts: Exploring Bone and Dental Age Assessment in Age Estimation Methods

Mariana Ferreira [1,2] and Francisca Alves Cardoso [1,3,*]

[1] Laboratory of Biological Anthropology and Osteological Human Remains (LABOH)—Centre for Research in Anthropology, NOVA University of Lisbon—School of Social Sciences and Humanities Branch (CRIA-NOVA FCSH), Av. de Berna 26 C, 1069-061 Lisboa, Portugal; marianabatistafer@hotmail.com (M.F.)

[2] Environmental and Human Biology, Faculdade de Ciências da Universidade de Lisboa (FCUL), 1749-016 Lisboa, Portugal

[3] Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory (IN2PAST)—School of Social Sciences and Humanities Branch (CRIA-NOVA FCSH), Av. de Berna 26 C, 1069-061 Lisboa, Portugal

* Corresponding author. E-mail: francicard@fcsh.unl.pt (F.A.C.)

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ABSTRACT: Age estimation (AE) is a fundamental aspect used to establish the biological profile of both living and deceased individuals. This study evaluates AE methods to determine if bone development (BD) methods yield similar results to dental development (DD) and whether methods using samples with similar geographic origins, socioeconomic status (SES), chronology, data specificity, and/or anatomical regions yield consistent results. We hypothesized that BD and DD methods differ in age estimations, although these differences would be minor when methods have similar variables. The sample consisted of 11 immature skeletons from the Hospital Real de Todos os Santos' collection (18th-century, Lisbon, Portugal) and applied 56 AE methods. The results were compiled into individual-based diagrams, facilitating both within- and between-individual comparisons, including stress-induced changes. This showed that BD methods tended to underestimate age compared to DD methods. BD methods closely aligning with DD methods were mainly based on individuals from lower to middle SES, focusing on areas like the iliac crest and medial clavicle. Findings also suggest that physiological stress might influence AE outcomes. This study emphasizes the importance of combining BD and DD methods alongside a detailed pathological and/or chronic stress assessment of human remains when estimating AE to minimize interpretative errors. This care applies to any discipline aiming to profile living or dead individuals, highlighting the importance of controlling for confounding variables, such as disease, in any AE estimation.

Keywords: Age estimation; Skeletal maturation; Dental development; Individual health

1. Introduction

Age estimation (AE) is a key component when establishing an individual's biological profile, alongside biological sex estimation and pathological assessment, in an array of disciplines in which human remains are a major source of information. Such disciplines include human biology, paediatrics, biological anthropology, bioarchaeology, human evolution, forensic anthropology, paleopathology, and many more [1–7]. In the last decades, the body of research on AE methods has been varied and numerous, largely driven by forensic anthropology's exponential growth and increased access to what are known as Identified osteological collections or reference collections. These collections have enabled the development of AE methods while controlling for biological, socio-cultural, economic, and other variables [8,9]. Despite notable progress and scientific contribution, challenges related to the performance and accuracy of these methods persist [6,10,11]. This study addresses these ongoing concerns and is rooted in central questions about AE: Do all AE methods perform equally for individuals within the same sample? do all methods yield consistent AE results when applied to a specific individual, or even when comparing different individuals from the same sample?

For methods to perform equally across all individuals within a sample, maturation rates must be similar for every individual. However, research has already suggested the existence of population-level differences in bone maturation due to a myriad of factors [12,13]. When we consider the human skeleton, these differences are not limited to the

sequence of maturational events (e.g., the order in which every epiphysis fuses) but also the timing of these events [14]. Initially, these differences in maturation rates were thought to be primarily driven by different genetic pools related to geographical factors. However, more recent studies have found them linked to far more complex factors beyond individuals and their genetics. Studies have concluded that these variations are more likely the result of individual responses to environmental influences, such as access to education, proper nutrition, socioeconomic status (SES), health care, occurrence of epidemics and/or exposure to pollutants and contaminants. While these factors are known to affect development, their impact is not linear, even within a single population: it varies between individuals [15–19]. It is also important to note that formal data about an individual's life is often unavailable when working with archaeological samples. This means that information about SES, periods of physiological stress, and other factors can only be inferred from the burial context and observable characteristics of the skeleton during analysis. It is also essential to notice that physiological stress does not only affect the linear growth of the skeleton; it can also lead to stress-induced bone changes (SIC), such as linear enamel hypoplasias (LEH), cribra orbitalia (CO), cribra humeralis (CH), cribra femoralis (CF), porotic hyperostosis (PH) and periosteal reactions (PR) [20–22]. A word of caution is also necessary when addressing disease-related bone changes: their interpretation requires a comprehensive and detailed analysis, and often enough, they are multifactorial in origin. For this reason, pinpointing a specific aetiology of the SICs mentioned above is challenging, leaving most of these causal links still unknown. For example, the connections between CO and PH with anemia have been extensively explored, with enough evidence to suggest that anemia is a strong potential cause for these SICs [23–29]. However, each case is unique, and it is crucial to study the distribution of SICs throughout the skeleton and perform rigorous differential diagnoses to assess the impact a condition could have had on bone development. Only after this exercise can one adequately assess the biological profile of the remains under analysis, fleshing out the interconnectivity between SIC and environment and its impact on bone development and, ultimately, age at death assessment. This raises a critical question: Should maturation be considered linear when using AE methods?

Bone maturation and growth occur in two major periods that alternate between each other: periods of saltation, in which linear growth is visible and measurable, and periods of stasis, during which no measurable growth of the individual occurs. However, the normal progression of these stages can be impaired by factors such as nutritional stress. In these situations, growth may cease, leaving the individual in a prolonged period of stasis. In long bones, a period of stasis results in the cessation of calcium deposition, which leads to a reduction or complete absence of linear growth. If proper nutrition is restored, development can resume to standard rates, allowing for a "catch-up growth" and a recovery of some of the lost growth potential [17,23]. On the other hand, if proper nutrition is not restored, malnutrition and/or subnutrition can become chronic. When this happens during a growth phase, the immune system's development may be compromised, leaving the individual in a permanent state of frailty. This can lead to chronic inflammation, infection, and permanent small stature (stunted growth) [23]. Several studies have also suggested that the timing of epiphyseal fusion is less affected by nutritional stress than the linear growth of long bones (primarily responsible for an individual's height). If an individual remains in a prolonged state of stasis, catch-up growth can only happen until the complete fusion of the epiphyseal plates. After this occurs, the potential for linear growth disappears. In this way, height can be considered an overall indicator of health and life quality within a given population [30–32]. A number of studies have also suggested that dental development (DD) and eruption are not as severely affected by external factors as bone development (BD). As a result, dentition is considered more reliable for estimating chronological age (CA) in immature individuals. Therefore, comparing the differences in AE between BD and DD may serve as a method to assess the influence of external factors on an individual's development and growth [23,30,33,34]. This raises another important question: if maturation does not always occur linearly, will AE methods based on dental and bone development provide similar results?

The relevance of the research proposed here lies in the increasing need to estimate age in both living and dead individuals. As previously stated, over the last few decades, AE methods have significantly increased in number due to various reasons, including the rising number of migrants and asylum-seekers. However, these methods must be tested to determine their applicability to each case in which age needs to be estimated. This can be achieved by comparing AE results provided by methods based on BD with those offered by DD. Since DD was shown to be less affected by external factors and exhibits low inter- and intra-population variation, AE results obtained from DD methods can work as a benchmark to compare with those provided by BD methods. If these estimates present consistent discrepancies, it may indicate that BD rates differ from those of the samples used to develop the BD methods applied. This analysis is complemented and enriched by analysing each method's characteristics (including samples' geographical origin, chronology, SES, and methodologies employed). This comparison may reveal trends among the BD methods that provided AE results similar to DD methods. To explore the above-mentioned, this study will run along two hypothesis tests:

- Intra-individual level hypothesis: We hypothesize that BD methods will yield different results compared to DD methods within the same individual. We expect that individuals showing the most notable discrepancies will also exhibit a higher number of SICs. To test this hypothesis, we present results from individuals UE.575 and UE.1008. The individuals in the sample are identified using their designated stratigraphic unit (Unidade Estratigráfica—UE) as per archaeological data.
- Inter-individual level hypothesis: We hypothesize that for different individuals with similar AE estimates based on DD methods, BD methods will perform differently. We anticipate these discrepancies to be more pronounced in individuals with a higher number of SICs. Results from individuals UE.1214 and UE.1296 are displayed to examine this hypothesis.

This research also explores the many questions outlined above. Note that the aim is clear—to evaluate AE methods to determine if bone development (BD) methods yield similar results to dental development (DD) and whether methods using samples with similar geographic origins, socioeconomic status (SES), chronology, data specificity, and/or anatomical regions yield consistent results. The aim is not to assess the methods' accuracy. We assumed that each method's accuracy level is the one reported per each original study. Hence, this research constitutes a unique contribution as it dives blindly into bone and teeth development, as research often does in forensic contexts and as per most archaeological and historical studies. By doing so, it pinpointed the many limitations of AE, and framed future studies. Most importantly, it raises further awareness of the implications of inaccurate AE in forensic investigations, which may lead to misidentifications and inappropriate legal outcomes. Although the impact may be less significant in archaeological and historical studies, it could still bias our understanding of past population demographics and their resilience in bottleneck events, such as diseases, wars, or other crises.

2. Methodological Approach

This research was developed by assessing the skeletal remains exhumed from an archeological context associated with the Hospital Real de Todos os Santos' (HRTS: 18th Century, Lisbon, Portugal) [35]. The overall collection comprises 17 individuals exhumed from nine primary graves, five containing multiple burials. Most of the individuals were classified as male (male = 9; female = 6; remaining undetermined). The collection age range comprised non-adults, two children estimated to have died at the ages of 6 to 12 years, and adults; the oldest most probably died at more than 30 years, but the vast majority were classified as being young adults [35].

Of the original collection, only individuals with incomplete bone maturation were selected for this study to test the hypothesis mentioned above: this sub-sample was composed of 11 individuals. Biological sex was only estimated in individuals with fully developed and fused os coxae to minimize the interpretative error associated with biological sex estimation in younger individuals [7,36,37]. Sex was then estimated based primarily on the morphology of the os coxae. If this was impossible, the skull was examined, followed by metric assessments [4]. In cases where none of these options were viable, biological sex was classified as indeterminate. As a result, four individuals were classified as indeterminate, four as female, and three as male.

This study's methodology for age-at-death assessment consisted of four distinct steps. The first step was a macroscopic analysis of the remains, in which every possible bone and teeth Age Indicator (AI) and its corresponding developmental stage were individually recorded. The next step involved selecting the different Age Estimation (AE) methods to be used in each skeleton. A total of 56 known AE methods were selected: 50 based on BD and 6 based on DD [38]. The publication years of these 56 methods ranged from 1989 to 2020, the latter being the year of the macroscopic assessment of the remains used in this study. Efforts were made to include methods based on samples from different geographical origins, periods, and SES, aiming to explore results and correlate these with those aspects of the methods. Additionally, the selected methods used different study materials (e.g., dry bone, X-ray, CT scan, *etc.*), anatomical regions (different individual bones or combinations of bones), and methodologies (e.g., measurements, morphological features, and/or epiphyseal union), aiming to be overarching of the various typologies of methods available for AE. Each BD and DD have individual contributions to AE; for example, BD has been proven to be more highly influenced by environmental stressors when compared to DD [17,23,30–34]; therefore, a combination of methods addressing both BD and DD aids in identifying various levels of development variability and implications on AE estimation. Also, by considering methods based on various measurements, morphological features, and/or epiphyseal union, we were able to explore methods-related AE variability and pinpoint future research avenues. For example, the majority of methods were developed in samples associated with European human osteological collections, eleven of which used samples from Portuguese human identified collections, namely the Luis Lopes Collection, curated

in the National Museum of Natural History and Science (Lisbon) and the University of Coimbra Identified Collections [38]. The majority of the BD methods ($n = 24$) were based on dry-bone assessment, which was in line with the approach undertaken in this research, and the remaining 26 BD methods used of CTScan ($n = 10$), X-ray ($n = 10$), and MRI ($n = 6$) analysis. The variability of anatomical features used for BD methods also matched the sample under study, as most methods could be used for all individuals under analysis. The combination of upper and lower limbs, either individually or joint, epiphyseal fusion assessment was recurrent (68%, $n = 34$), followed by methods (24%, $n = 12$) based on anatomical regions from the axial skeleton, *i.e.*, vertebral column (presacral and sacral vertebrae development), spheno-occipital synchondrosis, and the sternum. The remaining methods (8%) combined both axial and appendicular skeletal elements [38].

The third step of the methodological approach involved constructing individual-based diagrams. These feature each AE result obtained from the various methods used, facilitating comparison of the results and assessing results overlap and/or divergencies within and between individuals. Descriptive statistics were used to compare the parameters used by the BD methods, which provided AE results similar to those of the DD methods. A direct comparison between AE from BD and DD methods was not possible for individuals where dentition was not recovered. These cases were analyzed only after the individuals with available dentition had been assessed. This allowed us to establish a reference sub-set of five BD methods (BD subset) that showed the least discrepancies in AE results compared to the DD methods. These five BD methods were then used as a reference for comparison with the remaining BD methods in individuals without dentition. Finally, a visual analysis of SICs was conducted on the sample, examining features such as: Linear Enamel Hypoplasia (LEH), Porotic Hyperostosis (PH), Cribra Orbitalia (CO), Cribra Femoralis (CF), Cribra Humeralis (CH), and periosteal reactions (excluding those related to trauma). The results from this analysis were then compared with the findings from the previous step to determine if there was an overlap between the individuals presenting a higher number of SICs and those with notable discrepancies in their AIs.

We will present the results from the pooled total sample ($n = 11$ individuals) and the more relevant individual case studies that allowed us to explore the hypothesis under analysis, which were:

- Intra-individual level hypothesis: We hypothesize that BD methods will yield different results compared to DD methods within the same individual. We expect that individuals showing the most notable discrepancies will also exhibit a higher number of SICs. To test this hypothesis, we present results from individuals UE.575 and UE.1008.
- Inter-individual level hypothesis: We hypothesize that for different individuals with similar AE estimates based on DD methods, BD methods will perform differently. We anticipate these discrepancies to be more pronounced in individuals with a higher number of SICs. Results from individuals UE.1214 and UE.1296 are displayed to examine this hypothesis.

For a more comprehensive and extensive analysis of all the individual studies, please consult Ms Ferreira's dissertation [39].

3. Results

3.1. Total Sample

The composed graph (Figure 1) was created to provide a clearer understanding of the results. This graph represents a simple count of the number of stances (*i.e.*, the number of individuals in the sample) where the results of each BD method were similar to those of the DD or reference BD methods (BD subset). For example, the first method—Coqueugniot & Weaver, 2007—gave a similar AE result to either the DD methods or the BD subset in a total of 8 individuals from the sample. The method described by Cardoso (2008) was applied to seven individuals, among others. Notable, no method achieved similar AE results to either the DD methods or the BD subset across the entire sample, with the maximum observed value being 8.

These methods were further divided into two groups to identify their differences and similarities and determine which variables might influence their performance. This division was based on the BD methods that provided AE results more frequently aligned with the reference methods (DD methods and BD subset): Group_1—consisting of BD methods that matched BD subset methods in eight individuals (methods identified in darker blue, in Figure 1), and Group _2—which included BD methods that did match the BD subset and DD methods in seven individuals (methods identified in medium blue, in Figure 1).

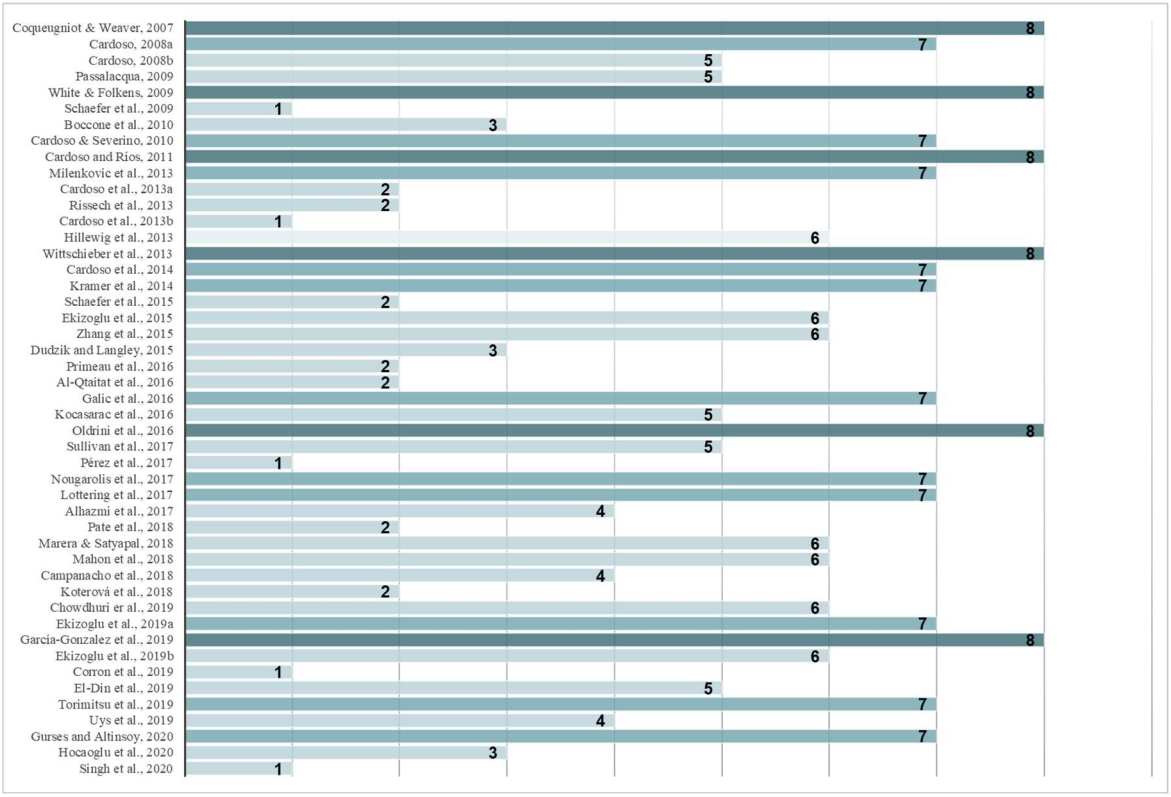

Figure 1. Bar graph showing the frequency of similar AE results between BD methods and reference methods (BD subset) across the sample. Dark blue bars represent BD methods that provided a similar AE to the reference methods in eight individuals. Medium-blue bars represent BD methods with similar estimates in seven individuals, and light-blue bars represent methods with similar estimates in six or fewer individuals (detailed reference of methods may be found in Ferreira and Alves-Cardoso 2024 [38]).

The characteristics of each method were summarized in a table to facilitate comparisons. Table 1 presents a segment of this table, focusing on the BD methods from Group_1. It includes information such as the methods' number, sample details (country and continent of origin, century, number of individuals in the sample (N), sex distribution—number of females, males, and indeterminates (F/M/I)—and the socioeconomic status (SES) of the samples as reported by the authors), study material, skeleton elements/areas examined and AE criteria employed. Following this, a descriptive statistics approach was applied to identify the most common characteristic among the BD methods and determine which factors might explain the improved performance of certain methods.

All BD methods in Group_1 (Table 1) used samples from Europe, with half of these samples originating from Portugal. The samples were dated between the 19th and 21st centuries and half of the methods employed samples characterized by either "Low" or "Low to Middle" socioeconomic status (SES). The remaining half either did not report SES data or lacked access to information on the samples' SES.

More than half of these methods used dry bone samples, with the most common methodology being evaluating epiphyseal union by stages. The most frequent were, specifically, a 3-stage union [40,41], a 5-stage union [42], and an 8-stage union [43]. In the Group_1 methods, the most commonly analyzed anatomical areas were the medial clavicle and the iliac crest (Table 1), whereas Group_2 (Table 2) included a larger number of BD methods, introducing a greater diversity to the results.

Table 2 presents the information regarding methods from Group_2. In this group, method-based samples originated not only from Europe (primarily from Portugal), but also from Asia (Turkey and Japan) and Oceania (Australia), dating between the 20th and 21st centuries, with more than half from the latter period. SES was classified as "Low", "Middle to High", and "Low to Middle", with "Low to Middle" being the most common. SES data was either unreported or unknown for more than half of these samples.

Table 1. Summary of the characteristics of the BD methods from Group 1.

Methods' Number	Country/ Continent	Century	*N* *	F/M/I *	SES *	Study Material	Skeleton Element/Area	AE * Criteria
1 [44]	Portugal/Europe	19th–20th	137	69/68/0	Low	Dry Bone	Cranium/Postcranial skeleton	3 stages epiphyseal union
5 [4]	*N*/*r* *	*N*/*r* *			*N*/*r* */Unknown	Dry Bone	Cranium/Postcranial skeleton	3 stages epiphyseal union
16 [45]	Germany/Europe	21st	566	253/313/0	*N*/*r* */Unknown	X-ray	Ilium/Lower Limb	8 stages epiphyseal union
28 [46]	France/Europe	21st	456	148/308/13	*N*/*r* */Unknown	CT scan	Sternum/Rib Cage	Morphology
42 [47]	Portugal/Europe	19th–20th	177	89/88/0	Low to middle	Dry Bone	Femur/Lower Limb	5 stages epiphyseal union

* *N*/*r* = not reported; *N* = number of individuals used in the method; F/M/I = Female, Male, Indetermined; SES = Social Economic Status; AE = Age Assessment.

The most frequently used study materials in Group_2 were dry bone, MRI and CT scan samples. All but one method used various stages of epiphyseal union, with the three and five stages being the most commonly employed. Additionally, all but one method focused on anatomical areas from the appendicular skeleton, with the medial clavicle being the most frequently analyzed anatomical area (Table 2).

Table 2. Summary of the characteristics of the BD methods from Group_2.

Methods' Number	Country/ Continent	Century	N *	F/M/I *	SES *	Study Material	Skeleton Elemento/Area	AE * Criteria
2 [41]	Portugal/Europe	20th	106	57/49/0	Low to middle	Dry Bone	Lower Limb	3 stages epiphyseal union
8 [48]	Portugal/Europe	20th	92	49/43/0	Low to middle	Dry Bone	Upper Limb/Lower Limb	3 stages epiphyseal union
11 [49]	Serbia/Europe	20th–21st	67	25/42/0	Middle to high	Human cadavers	Clavicle/Scapular girdle	Morphology
17 [50]	Portugal/Europe	20th	191	90/101/0	Low to middle	Dry Bone	Sacrum/Vertebral Column	4 stages epiphyseal union
18 [51]	Germany/Europe	21st	290	124/166/0	*N*/*r* */Unknown	MRI	Tibia/Lower Limb	8 stages epiphyseal union
26 [52]	Italy/Europe	21st	446	212/234/0	*N*/*r* */Unknown	X-ray	Lower Limb	3 stages epiphyseal union
31 [53]	France/Europe	21st	232	109/123/0	*N*/*r* */Unknown	CT scan	Scapula/Scapular girdle	5 stages epiphyseal union
33 [54]	Australia/Oceania	21st	524	241/283/0	*N*/*r* */Unknown	CT scan	Ilium/Pelvic grid	7 stages epiphyseal union
41 [55]	Turkey/Asia	21st	428	188/240/0	Low	MRI	Humerus/Upper Limb	5 stages epiphyseal union
46 [56]	Japan/Asia	21st	207	79/128/0	*N*/*r* */Unknown	CT scan	Clavicle/Scapular girdle	9 stages epiphyseal union
48 [57]	Turkey/Asia	21st	598	231/367/0	*N*/*r* */Unknown	MRI	Lower Limb	5 stages epiphyseal union

* *N*/*r* = not reported; *N* = number of individuals used in the method; F/M/I = Female, Male, Indetermined; SES = Social Economic Status; AE = Age Assessment.

Table 3 shows the different SICs that were observed across the total sample, with the most common SIC observed being PR of varying degrees, with both woven and/or lamellar bone observed in 100% of the cases. These reactions had a higher incidence of diaphysis in the long bones, particularly in the lower limbs. LEH was found in every individual with preserved dentition, showing a higher incidence in the lower and upper incisors and canines. Approximately 45% of the sample exhibited active CF, while only two individuals showed signs of active CH (Table 3). No individuals were without SIC (Table 3), indicating that every individual exhibited pathological changes suggestive of physiological stress during their lifetime. The individuals with the highest number of observed SIC were UE.575, UE.1008, UE.1214 and UE.1296, and their results are presented below.

Table 3. Table representing the number of SIC present in every individual of the sample: "–" = absence of SIC; "+" = presence of SIC.

UE *	LEH *	PH *	CO *	CF *	CH *	PR *
UE.573		–	–	+	–	+
UE.575	+	–	–	–	+	+
UE.872		–	–	–	–	+
UE.1008	+	–	–	+		+
UE.1214	+	–	–	+	+	+
UE.1296	+	–	–	+	–	+
UE.1313		–	–	–		+
UE.1314	+	–	–	–	–	+
UE.1406	+	–	–	+	–	+
UE.1419		+	–	–	–	+
UE.1429	+	–	–	–	–	+

* Stratigraphic Unit (UE), Linear Enamel Hypoplasia (LEH), Porotic Hyperostosis (PH), Cribra Orbitalia (CO), Cribra Femoralis (CF), Cribra Humeralis (CH), Periostitic Reaction (PR).

3.2. Case Study UE.575

A visual diagram similar to that of Figure 2 was plotted for every individual of the sample to facilitate the understanding of the results obtained. The X-axis represents the age (in years) estimated by each of the BD methods applied (represented in the bars in lighter blue, and coded by number in the Y-axis—for a correspondence between the methods' code and its respective reference, check the link in Supplementary Materials). For example, in the diagram shown in Figure 1, Method 1 provided an age estimate ranging from 12 to 16 years for the individual UE.575. For individual UE.575, the intersection of DD methods yielded an overall age estimate of 15.5 years. This intersection established the age interval to which all BD methods were compared. Biological sex could not be determined due to the lack of fusion in the three bones of the *os coxae*. Out of the initial BD methods selected, only forty-two could be applied. Of these, only twenty methods provided an AE that either wholly or partially overlapped with the estimate given by the DD methods (this is represented in Figure 2 by the number of light blue bars that intersect the black rectangle, which depicts the AE obtained from the crossover of each DD method), representing 47.6% of the applied methods.

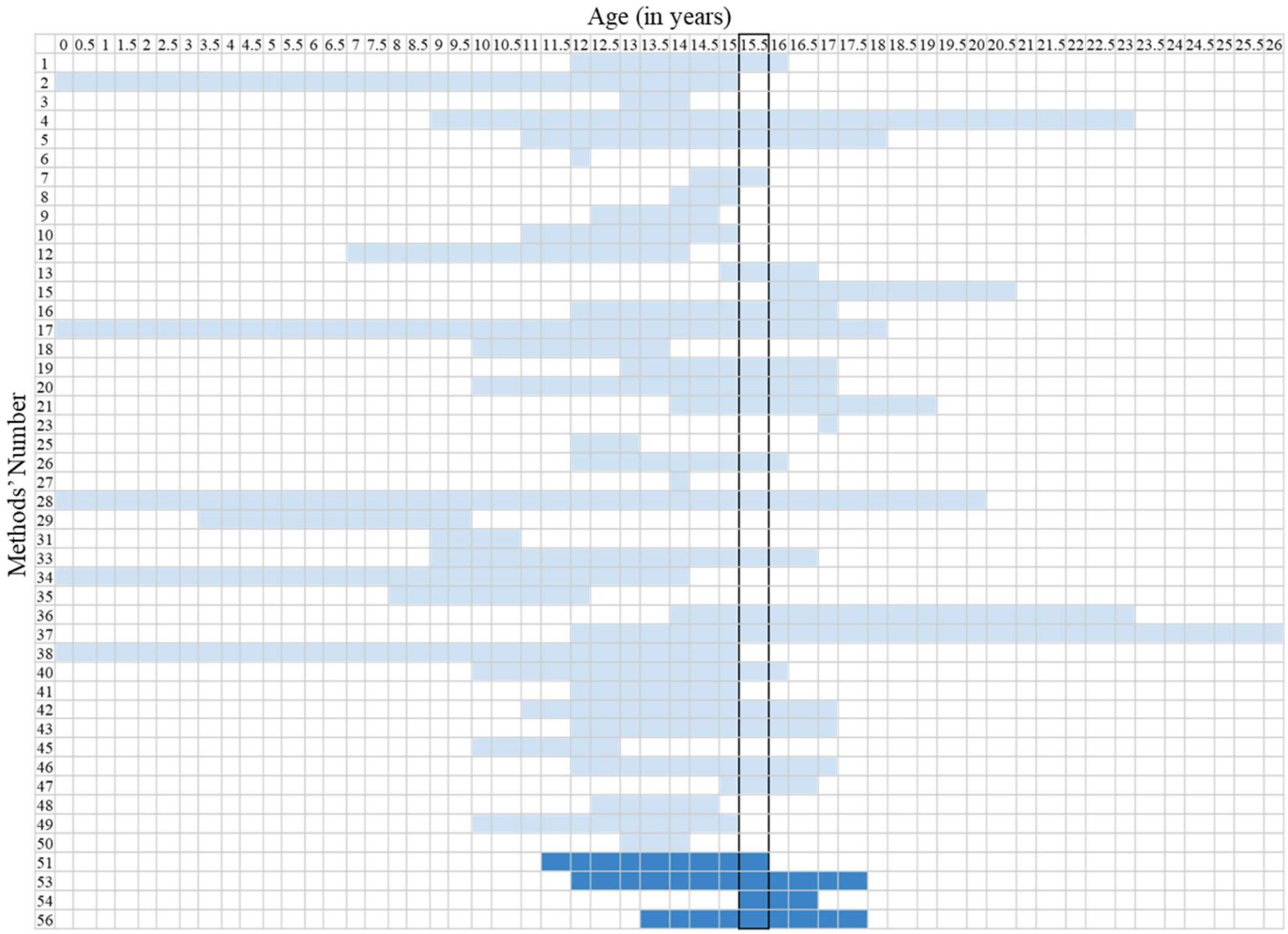

Figure 2. Diagram showing the AE results provided by each method for individual UE.575. The horizontal axis represents the age estimates (in years) produced by each method, while the vertical axis indicates the method number. The darker blue bars depict the AAD estimates from the DD methods, and the lighter blue bars represent those from the BD methods. The black outline highlights the age range in which all DD methods overlap. For a correspondence between the methods' numbers and their detailed reference, please check Ferreira and Alves-Cardoso 2024 [38].

Table 4 provides a more comprehensive view of the information from these BD methods, similar to Tables 1 and 2. As described in the section above, a descriptive statistical approach was used to identify the most common characteristics among the BD methods and to determine the factors that might explain the improved performance of the BD methods that produced AEs similar to the DD methods. This approach was also applied to each individual human skeletal remnant in the sample, including those described below.

Table 4. Summary of the characteristics of the BD methods from individual UE.575.

Methods' Number	Country/Continent	Century	*N* *	F/M/I *	SES *	Study Material	Skeleton Area	AE * Criteria
1 [44]	Portugal/Europe	19th–20th	137	69/68/0	Low	Dry Bone	Cranium/Postcranial skeleton	3 stages epiphyseal union
4 [58]	USA/North America	20th	634	246/388/0	*N*/*r* */Unknown	Dry Bone	Sacrum/Vertebral Column	Morphology
5 [4]	*N*/*r* *	*N*/*r* *			*N*/*r* */Unknown	Dry Bone	Cranium/Postcranial skeleton	3 stages epiphyseal union
7 [59]	Egypt/Africa	2300–1750 BCE	130	*N*/*r*	Low to middle	Dry Bone	Upper Limb/Lower Limb	Measurements
13 [60]	Spain/Portugal/Britain/Europe	19th–20th	346	173/173/0	Low to middle	Dry Bone	Upper Limb/Lower Limb	Measurements
16 [45]	Germany/Europe	21st	566	253/313/0	*N*/*r* */Unknown	X-ray	Ilium/Pelvic girdle	8 stages epiphyseal union
17 [50]	Portugal/Europe	20th	191	90/101/0	Low to middle	Dry Bone	Sacrum/Vertebral Column	4 stages epiphyseal union
19 [61]	USA/North America	*N*/*r*	453	189/264/0	Low to high	X-ray	Upper Limb	1–4 stages of epiphyseal union
20 [62]	Turkey/Europe-Asia	21st	503	141/362/0	Low	CT scan	Clavicle/Scapula girdle	5 stages epiphyseal union
21[63]	China/Asia	21st	1777	717/1060/0	Low to high	X-ray	Ilium/Pelvic girdle	8 stages epiphyseal union
26 [52]	Italy/Europe	21st	446	212/234/0	*N*/*r* */Unknown	X-ray	Lower Limb	3 stages epiphyseal union
28 [46]	France/Europe	21st	456	148/308/13	*N*/*r* */Unknown	CT scan	Sternum/Rib Cage	Morphology
33 [54]	Australia/Oceania	21st	524	241/283/0	*N*/*r* */Unknown	CT scan	Ilium/Pelvic girdle	7 stages epiphyseal union
36 [64]	South Africa/Kenya/Africa	*N*/*r* *	1605	800/805/0	Low to middle	X-ray	Clavicle/Scapula girdle	5 stages epiphyseal union
37 [65]	South Africa/Africa	20th	211	110/101/0	*N*/*r* */Unknown	Dry Bone	Clavicle/Scapula girdle	3 stages epiphyseal union
40 [66]	India/Asia	21st	157	65/92/0	*N*/*r* */Unknown	X-ray	Ilium/Pelvic girdle	8 stages epiphyseal union
42[47]	Portugal/Europe	19th–20th	177	89/88/0	Low to middle	Dry Bone	Femur/Lower Limb	5 stages epiphyseal union
43 [55]	Turkey/Europe-Asia	21st	395	173/222/0	Low	MRI	Humerus/Upper Limb	5 stages epiphyseal union
46 [56]	Japan/Asia	21st	207	79/128/0	*N*/*r* */Unknown	CT scan	Clavicle/Scapula girdle	9 stages epiphyseal union
47 [67]	South Africa/Africa	21st	974	513/461/0	Low to high	X-ray	Cervical vertabrae/Vertebral Column	5 stages epiphyseal union

* *N*/*r* = not reported; *N* = number of individuals used in the method; F/M/I = Female, Male, Indetermined; SES = Social Economic Status; AE = Age Assessment.

The BD methods applied used samples primarily from Europe (mainly Portugal) and Asia (predominantly Turkey), with only one method using a sample from Oceania (Australia) (Table 4). The sample chronologies ranged from 2300–1750 BCE to the 21st century, with half of the samples dating from the current century. The most commonly described SES were "Low to Middle", "Low to High", and "Low". Dry bone was the most frequently used study material. A total of 78.9% of the methods relied on different stages of epiphyseal union to do the AE, with the most common being the 3, 5, and 8 stages of epiphyseal union. The most commonly used anatomical areas for BD methods were the medial clavicle and iliac crest. In total, 30% of the methods applied to individuals UE.575 used a combination of skeletal elements, while the remaining 70% focused on a single element.

Individuals UE.575 exhibited three types of SIC: LEH, CH, and PR (Table 3). LEH was observed in both the maxillary and mandibular dentition, with the most prominent signs in the incisors and canines. Every permanent tooth displayed at least one LEH, including the unerupted third molars, where the roots were still open at the time of death. PR, in the form of woven and lamellar bone, was noted on the long bones of both the upper and lower limbs.

3.3. Case Study UE.1008

In the case of the individual's remains UE.1008, Figure 3 shows that the intersection of the DD methods (represented by the dark blue bars) applied provides an age range between 5.5 and 6 years. This intersection established the age interval to which all of the BD methods were compared. Biological sex could not be determined due to the lack of fusion of the three bones of the *os coxae*. Out of the initial fifty BD methods selected, only twelve could be applied to this individual. Of those, only five methods produced an AE that either partially or completely aligned with the estimate from the DD methods (as observed in Figure 3, by the number of light blue bars that cross the black rectangle, which depicts the AE obtained from the crossover of each DD method), representing 41.7% of the applied methods.

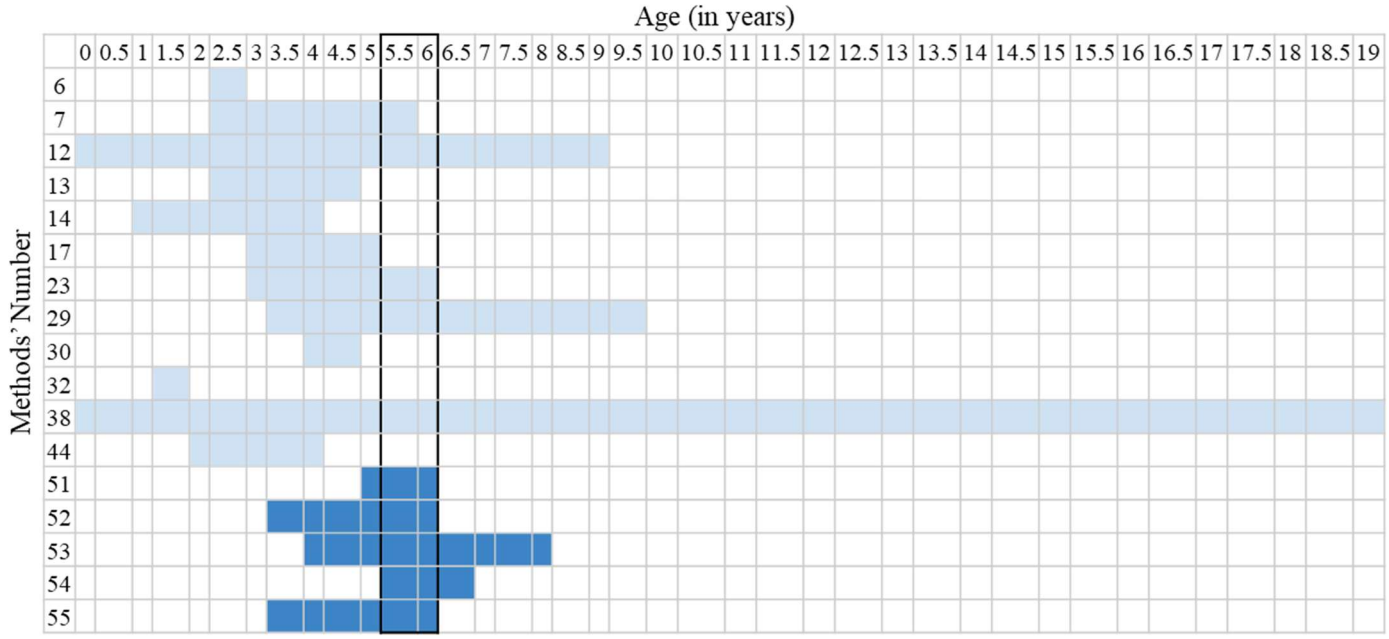

Figure 3. This diagram illustrates the AE results for individuals UE.1008 as provided by each method. The horizontal axis displays the age estimates (in years) generated by the various methods, while the vertical axis shows the method numbers. Darker blue bars indicate the AAD estimates from the DD methods, whereas lighter blue bars reflect those from the BD methods. The black outline indicates the age range where all DD methods converge. For a correspondence between the methods' numbers and their detailed reference, please check Ferreira and Alves-Cardoso 2024 [38].

Table 5 outlines the characteristics of each of the five BD methods that produced AEs similar to those of the DD methods. It includes details about the sample, study material, skeleton elements, and AE criteria in a format similar to the tables described above. Three of these methods used European samples (two from Portugal and one from Denmark), while the remaining two methods were based on samples from Africa (Egypt) and Oceania (Australia) (Table 5). The sample chronologies ranged from 2300–1750 BCE to the 21st century, with the 20th century being the most common. SES was described as either "Low" or "Low to Middle". The most commonly used study material for BD methods was dry bone samples. Of the methods applied, 40% relied on metric traits of the long bones, while the remaining 60% used

different stages of epiphyseal union, specifically the 3 and 6 stages (Table 5). Additionally, 80% of the methods focused on a combination of different anatomical areas from the appendicular skeleton, most commonly involving a combination of long bones from both the upper and lower limbs.

Table 5. Summary of the characteristics of the BD methods from individual UE.1008.

Methods' Number	Country/ Continent	Continent	Century	*N* *	F/M/I *	SES *	Study Material	Skeleton Element/Area	AE * Criteria
7 [59]	Egypt/Africa	Africa	2300–1750 BCE	130	*N*/*r*	Low to middle	Dry Bone	Upper Limb/Lower Limb	Measurements
12 [68]	Portugal/Europe	Europe	20th	148	69/79/0	Low to middle	Dry Bone	Lower Limb	6 stages epiphyseal union
23 [69]	Denmark/Europe	Europe	11th–16th	183	0/0/183	Low	X-ray	Upper Limb/Lower Limb	Measurements
29 [70]	Australia/Oceania	Oceania	21st	562	270/292/0	*N*/*r* */ Unknown	CT scan	Lower Limb	3 stages epiphyseal union
38 [71]	Portugal/Europe	Europe	20th	68	35/33/0	Low to middle	Dry Bone	Sternum/Rib Cage	3 stages epiphyseal union

* *N*/*r* = not reported; *N* = number of individuals used in the method; F/M/I = Female, Male, Indetermined; SES = Social Economic Status; AE = Age Assessment.

The remains of the individual UE.1008 exhibited three types of SIC: PR, LEH, and CF (Table 3). LEH was identified in the mandibular dentition, affecting both deciduous and permanent teeth (which were unerupted and had open roots), with more pronounced expression in the permanent teeth. CF was observed only in the right femur, as the left femur was not recovered. PR, primarily characterized by microporosity and/or woven bone, was present in the diaphysis of most long bones from both the upper and lower limbs, as well as in the vertebral bodies and the pubis, ilium, and ischium.

3.4. Case Study UE.1214 and UE.1296

The remains of the individuals UE.1214 and UE.1296 provided exciting results, exemplifying the variability of BD between individuals. The individual UE.1214 was classified as male, and the individual UE.1296 as female. For both individuals, AE was estimated to be ≥21 years based on DD methods (represented in Figure 4, on each of the diagrams by the intersection of the dark blue bars for both individuals). Out of the initial fifty BD methods used in the present study, forty-three could be applied to individual UE.1214. Of these, only twenty-four provided an AE similar to the one obtained from the DD methods, representing 55.8% of the applied methods. In the case of the individual's remains UE.1296, thirty-five BD methods were applicable, and twenty-one yielded an AE that coincided (wholly or partially) with the DD methods, corresponding to 60% of the methods applied. These results can be observed for both cases in Figure 4, by the number of light blue bars that cross the black rectangle,which depicts the AE obtained from the crossover of each DD method for each of the individuals. Although the AE results were similar for both individuals when using DD methods, the diagrams for these individuals reveal that identical BD methods produced different estimates for each one (Figure 4). Most of the methods applied to individual UE.1214 were also applied to individuals UE.1296. Yet, their diagrams differ significantly (in both cases, however, the methods falling outside of the intersection between the DD methods tended to underestimate age). This indicates that the same methods produced different AEs to each individual, highlighting important intra-populational differences that must be accounted for when estimating age. A clear example is method 5, which gave an AE of 14–15 years for an individual UE.1214 but yielded an AE of 22 years for an individual UE.1296. This pattern was observed across several of the methods applied to these individuals.

To explore the factors that may influence the methods producing age estimates similar to the DD methods, relevant data were compiled in a table similar to those described above. Based on this, Table 6 was created to highlight the most prominent results for each parameter for both individuals. Despite the various methods used, upon closely examining the parameters of the BD methods that yielded similar AE results to the DD methods, we observed these share identical criteria: (Table 6).

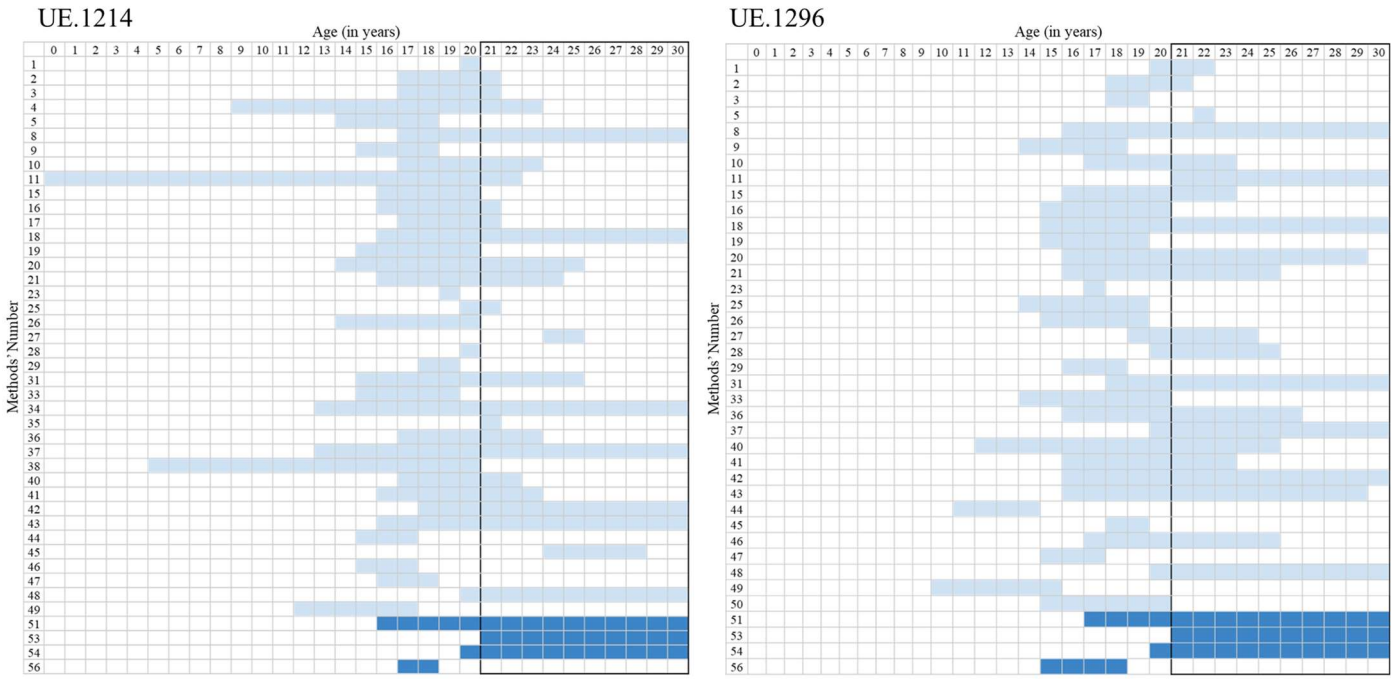

Figure 4. Diagram representing the AAD estimate provided by each method applied to individuals UE.1214 (on the **left**) and UE.1296 (on the **right**). On the horizontal axis, the numbers represent the age estimate (in years) provided by each method. The vertical axis represents each method's number. The AAD estimate obtained from the DD methods is represented by the darker blue bars, while the light blue bars represent the AAD estimate obtained from the BD methods. The black contour marks the age interval in which each DD method overlaps. For a correspondence between the methods' numbers and their detailed reference, please check Ferreira and Alves-Cardoso 2024 [38].

Table 6. Summary of the characteristics of the BD methods from individuals UE.1214 and UE.1296.

***N.* of Methods**	**UE.1214**		**UE.1296**	***N.* of Methods**
6	Portugal	**Country**	Portugal	5
5	Turkey		Turkey	5
13	21st	**Chronology**	21st	12
3	Low	**SES ***	Low	4
7	Low to middle		Low to middle	5
11	*N*/*r* */Unknown		*N*/*r* */Unknown	9
8	Dry Bone	**Study Material**	Dry Bone	7
5	MRI		MRI	5
4	CT Scan		CT Scan	5
6	3 stages epiphyseal union	**AE * criteria**	3 stages epiphyseal union	6
8	5 stages epiphyseal union		5 stages epiphyseal union	7
4	Medial Clavicle	**Skeletal Element**	Medial Clavicle	6
3	Iliac Crest		Iliac Crest	2

* *N*/*r* = not reported; *N* = number of individuals used in the method; F/M/I = Female, Male, Indetermined; SES = Social Economic Status; AE = Age Assessment.

The remains of the individual UE.1214 had the highest number of observed SICs, including LEH, PR, CF, and CH (Table 3). In comparison, three types of SICs were identified in individual UE.1296: LEH, CF, and PR (Table 3). LEH was present in both individuals, affecting both mandibular and maxillary dentition, with multiple lines visible on the incisors and canines and at least one line on each molar. PR was observed along the diaphysis of the long bones in both the upper and lower limbs in the form of lamellar bone.

4. Discussion

Overall, the results showed that BD methods tend to underestimate age at death compared to the DD methods. This supports the argument that bone and dental development do not progress at similar rates across populations or even between individuals of the same population, with external factors potentially influencing these discrepancies. For example, in individuals UE.1214 and UE.1296, the DD methods provided similar AE results (equal to or greater than

21 years). However, the AE results from the BD methods varied significantly between these two individuals (Figure 4). This suggests they experienced different developmental rates despite likely belonging to the same archaeological population. It is also necessary to consider that the individual UE.1214 was classified as male and the individual UE.1296 as female; hence, male/female differences in bone maturation may also be at play as female individuals reach puberty earlier than male individuals, experiencing more rapid changes in bone [72–74]. These findings highlight the importance of considering individual variation, as not all AE methods will yield consistent results, even within a single sample.

The prevalence of SIC further supports the results found and the need to control for external factors of bone development, including diseases and episodes of stress, which can impact a person's physiological development. Every individual with preserved dentition exhibited at least one LEH on the upper and lower incisors and canines, which are well-known indicators of systemic physiological stress. Additionally, all individuals displayed varying degrees of PR at the time of death, mainly affecting the long bones of the upper and lower limbs. CF was the second most common SIC observed. The presence of these stress indicators aligns with the low SES estimated for this sample, based on its social and historical context [35], further emphasizing the need to contextualize any human adequately remains when the aim is to assess as accurately as possible, age at death. That not being a possibility, any age assessment needs to be on the side of caution, for example, by providing a broader chronological age estimation rather than limiting age assessment to 5 years categories or, rather than offering a chronological age, advocating for qualitative age classifications such as child, young adult, adult, and others. While this approach may work well for studying human remains in archaeological contexts, it could be problematic in forensic cases, where the goal is to estimate the missing person's age as accurately as possible. For this reason, age estimation methods continue to invest in new approaches such as tooth cementum development and machine learning [75,76].

Our results also showed an association between the individuals with a higher number of age indicators (AI) discrepancies and those presenting a greater number of SICs. This pattern is particularly evident in the four case studies selected to be part of this article, *i.e.*, individuals UE.575, UE.1008, UE.1214, and UE.1296. However, it is essential to note that all individuals in the sample exhibited some level of AI discrepancies, even if to a lesser degree, and every individual had at least one SIC observable during the analysis. For this reason, the entire sample may showcase biased results due to illness and/or physiological stress, having shared similar stress levels. The archaeological context in which the sample was exhumed was associated with a hospital. For this reason, the likelihood of individuals being ill at the time of death is high.

Another observation is that we need to consider the sample's origin. Its provenance is associated with the Hospital Real de Todos os Santos' collection, and the sample chronology was inferred to be the 18th Century. Although we could assume that it was most likely composed of people of Portuguese origin, the fact is that Lisbon has long been a metropolis with a convergence of people of various geographies and origins. This is relevant when discussing the methods-based samples and inter-population biases. Although many of the more reliable BD methods (*i.e.*, those that provided the AE results most consistent with the reference methods) were based on Portuguese samples, a significant number of these methods also included samples from other geographical regions, including not only other European countries but also Asian regions like Turkey. As the results found in our research had wide AE discrepancies, we could argue that creating population-specific methods may not necessarily lead to more accurate or reliable results. However, and as stated above, at this point, we could not pinpoint the origin and/or geographic ancestry of the sample used in our analysis: we assumed it would be Portuguese in origin, which may warrant critique.

On another note, alongside disease, socio-economic status (SES) is another significant variable that impacts a person's development. SES is directly linked to an individual's access to essential resources such as healthcare, education, and proper nutrition. Growth and development can be significantly impacted when these factors are subpart due to a low SES—particularly common in low-income regions [30,46,50,58,61,64,77–80], parts of Africa and Asia, where unequal distribution of resources and limited access to food and healthcare result in higher rates of developmental delays and stunting. As proper nutrition is a critical element in ensuring adequate growth. However, undernutrition and/or malnutrition impact more than skeletal linear growth. Research by various authors suggests that prolonged exposure to inadequate nutrition increases the risk of developing chronic diseases, such as osteoporosis, cardiovascular conditions, and diabetes, leaving individuals more vulnerable and in a higher state of frailty that can last throughout their lifetime [81–84]. This aligns with the findings of the present study. Among the BD methods that provided AE results consistent with the reference methods, the most common SES descriptions were "Low" and "Low to middle". This may suggest that individuals from these samples experienced social distress, potentially including undernutrition and/or malnutrition, which may have impaired expected normal growth. The SES of individuals from the HRTS sample is similarly estimated as "Low", based on the hospital's historical and social context [35,85]. Given their similar socio-

economic conditions, this raises the possibility that the developmental rates of the samples used in the BD methods were comparable to those of the HRTS individuals. However, as we deal with an archaeological context, any interpretation needs to be carefully weighed, as social complexities may significantly strain human development. These are not limited to access to resources, but may also include issues related with migration. What we assess in an archaeological context is a snapshot at the time of death; much of the information is certainly missing, especially in the absence of documented data. There are added limitations related to sample preservation that cannot be ignored, as they will limit the selection of methods usable to AE, rendering its assessment less reliable [23–34,86–88].

Finally, another important finding is that most of the methods that provided AE results similar to those of the DD methods were based on a single bone, particularly the fusion of the medial clavicle epiphysis, proximal humerus, or iliac crest. These are among the last AIs to fully develop in the human skeleton and proved to be more reliable for AE in the individuals from this sample, especially when compared to DD methods. This suggests that methods relying on a single anatomical region may sometimes be more dependable than those combining multiple regions. A more accurate estimation may be achieved by applying multiple methods, each focusing on a specific anatomical area.

None of the fifty BD methods used in this study included an assessment of the SICs present in the samples, focusing instead on excluding only gross pathological cases (such as tumors or fractures). However, the present study suggests that incorporating SIC assessment is crucial for improving the reliability of age assessment.

5. Conclusions

The study presented here has allowed us to explore the proposed hypothesis, emphasizing the need for a cautionary approach when the aim is to estimate a chronological age at death based on human remains. This cautionary tale is valid regardless of the practical and disciplinary field in which the assessment of the remains is included: whether forensic anthropology, biological anthropology, bioarchaeology, or other—the key should always be to consider human variability and confounding variables able to impact human development. This was clear with the overall results and case studies, which were crucial to test the proposed hypothesis. At the intra-individual level, the AE for individuals UE.575 and UE.1008 showed that those with the highest number of discrepancies between DD and BD methods also had the greatest variety of SICs, highlighting the fact that physiological stress may have a significant impact on growth, biasing AE. On the other hand, at the inter-individual level, results indicated that for individuals with similar AE inferred with DD methods, the same BD methods showed considerably different behaviors. These discrepancies were particularly pronounced in individuals with a higher number of SICs, which once more point out the need for a holistic approach when exploring AE, in which bone maturation, dental development, and presence/absence of disease/physiological related bone and teeth changes are considered when assessing the remains. This care also states that human remains assessment combines multiple disciplines and that the assessment should not be limited to biological profiling (*i.e.*, sex, age, stature, ancestry/variability) but also include pathological profiling. Which, in turn, demands professional training and experience from those working in the field and laboratory.

Overall, the study suggests that different individuals may exhibit different developmental rates that are potentially influenced by the presence of SICs, which could indicate physiological stress and developmental disturbances of varied causes. These results highlight the importance of considering individual variation in age estimates (AE), as outliers can emerge even in small samples like the HRTS. Such outliers are often key to understanding underlying growth patterns influenced by disease or other factors. Additionally, methods that yielded more reliable results tended to have broader age ranges, making their estimates less precise but ultimately more accurate, which validates the need to compromise. And although this latter approach is acceptable when studying/exploring human remains from archaeological and historical contexts, it raises many issues in forensic and other contexts, in which the aim is to contribute to persons/remains positive/possible identification. Given the methods currently available for age-at-death estimation and their tested reliability, further research is needed to improve these techniques. New methodological approaches, such as those related to Machine Learning, may provide additional insights and aid in a more holistic individual- and populational-based approach to AE.

Supplementary Materials

Supplementary information on the BD and DD methods used in this research may be found at: Ferreira, M., & Alves Cardoso, F. (2024). Bone Development and Dental Development methods references [Data set]. Zenodo. https://doi.org/10.5281/zenodo.13996709.

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Author Contributions

Conceptualization, M.F. and F.A.C.; Methodology, M.F. and F.A.C.; Software, M.F.; Validation, M.F. and F.A.C.; Formal Analysis, M.F. and F.A.C.; Investigation, M.F.; Resources, F.A.C.; Data Curation, M.F.; Writing—Original Draft Preparation, M.F.; Writing—Review & Editing, F.A.C.; Visualization, M.F.; Supervision, F.A.C.; Project Administration, F.A.C.; Funding Acquisition, F.A.C.

Ethics Statement

This research was developed upholding and following the American Association of Biological Anthropology ethical guidelines and the British Association for Biological Anthropology and Osteoarchaeology (BABAO) Code of Ethics and Code of Practice for the analysis and handling of human remains. The research was carried out in close collaboration with institutional custodians of the collection of whom the remains belong.

Informed Consent Statement

Not applicable, as we may argue that human remains exhumed from archaeological context cannot provide consent; however, we would like to acknowledge the privilege to have had the opportunity to study the human remains that led to this research, further recognizing their contribution to the scientific development of the disciplines involved in this study as well as the historical context of the Hospital Real de Todos-os-Santos, in Lisbon, Portugal. We would also like to highlight the significance of human remains as a legacy from past populations and their importance as cultural heritage.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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