The feasibility of the adult age estimation 3D-CBCT method on ancient human remains

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ABSTRACT

The age estimation of skeletal remains still represents a central issue for the reconstruction of the so-called "biological profile," but mostly for reconstructing investigation. This research aims to verify the feasibility of the adult age estimation method developed on living people by Pinchi et al. (2015 and 2018), for estimating the age at the death of 37 subjects from ancient populations found in two different Italian necropoleis of archaeological interest (Mont'e Prama and Florence, X-IX century B.C and V-VI century A.D respectively). The method is conservative and based on a geometrical approximation of dental volumes of the upper central left incisors on CBCT scans. The statistical distribution of the age and errors followed the Bayesian approach proposed by Sironi et al. (2018) applying the *"a priori"* values according to the estimates/classification obtained with anthropological methods (morphological). Results show higher accuracy for Mont'e Prama remains than for the Florentine sample due to the different characteristics of the two ancient populations (estimates varying from 18.4 up to 28.7 years with a maximum error of 6,14 years for Mont'e Prama, and from 15.88 up to 43.37 years with a minimum error of 1 year up to a maximum error of 7,85 years for Florence). The method proposed and validated on modern living people can represent a reliable tool for estimating the age of ancient human remains with a significant palaeodemographic value for archaeologists/anthropologists. Mont'e Prama sample could be defined as a homogenous group of males aged around 20-30 years, probably warriors, soldiers, or athletes; the Florentine sample as an inhomogeneous group of males and females from different families buried all together in a small area out of the city due to the probable occurrence of a special healthy emergency in the city.

INTRODUCTION

Age estimation is one of the main goals of the personal identification investigation, relevant for living subjects, single and multiple deaths, [1-14] but also for human remains of anthropological/archaeological interest. [15-16]

In the archaeological field, the age estimation of skeletal remains still represents a central issue not only for the reconstruction of the so-called 'biological profile,' but also for bioarchaeological investigations into the living conditions of ancient populations, the pathologies that may have led to death, and any other useful data regarding the composition and characteristics of the studied population (palaeodemography).[17-18]

Furthermore, the age estimation procedure is complicated by adulthood due to the completion of skeletal and dental maturation and the need to apply the regressive and pathological skeletal/ dental features affected by high variability. Teeth reveal a wide inter-individual variability due to different living conditions, the incidence of pathologies or para-functions, and personal habits which may have caused significant and specific changes in tooth morphology and anatomy over the years.[19]

On the other hand, ancient skeletal remains often offer limited available evidence, but represent archival interest for paleoanthropological nature museums. The museum value of ancient remains pursues the development and the application of conservative methods based on radiographic examination of teeth for estimating individual age at death.[20]

The most applied dental techniques for age estimation are based on the direct evaluation of the macroscopic morphological changes in adult dental elements such as occlusal wear,[21-22] usually highly variable since influenced by both exogenous and endogenous factors, or dentin translucency, root resorption, and periodontal attachment loss,[23-27] physiological variations that occur in healthy teeth. These latter techniques are not suitable for archaeological finds due to their invasiveness, as they require the extraction or sectioning of dental elements. Other common methods rely on bi-dimensional radiographic measurements of dental pulp cavity reduction.[28-30]

However, when applied individually to estimate adulthood, none of these methods provide an accuracy better than 10-15 years. Therefore, it is often advisable to use a combination of different estimation methods to improve the confidence interval and increase the precision of adult age determination.[18, 31-34]

Since the most reliable dental parameter for estimating adulthood appears to be the deposition of secondary dentin,[35] the introduction of the CBCT (Cone Beam Computed Tomography) in the common clinical practice has created new valid opportunities to obtain three-dimensional tooth radiographs to measure this phenomenon with absolutely non-invasive techniques.[36-37]

Some methods addressed to estimate the adult dental age are based on the ratio between 3D dental volumes using computed tomography: dental pulp chamber and the entire tooth volume (mineralized tissues) of various types of single-rooted teeth. Someda et al.[38] applied micro-CT scans to reconstruct three-dimensional structures of enamel, dentin, and pulp cavity of mandibular central incisors from subjects aged 12-79 years. They calculated the ratio between the pulp cavity and the whole tooth volumes, obtaining a high correlation between the pulp chamber narrowing and the increasing age in both sexes, but better accuracy in females. Similarly, Agematsu et al.[39] applied the micro-CT methodology and found a significant degree of pulp volume decrease between age-cohorts of the forties, fifties, and sixties years. Star et al.[40] developed a method based on CBCT scans of mono-rooted teeth (incisors, canines, and premolars) to calculate the ratio between the tooth volumes, requiring an automatic process of separation and segmentation of the tooth scans (applying a grey scale) to reconstruct and measure the entire 3D image. They found a difference between the real volume of the pulp and the volume measured with the software on the 3D structure of 21%, and 16% between the real tooth volume and the measured one. The entire procedure took on an average of 3 hours per tooth. Boedi et al.[41] improved the segmentation method by focusing on the crown part of mono-rooted teeth measuring three parameters (enamel volume, dentine volume, and pulp chamber volume) and obtaining a pulp to dentine volume ratio and an enamel to dentine volume ratio. They concluded that the highest correlation can be found in the maxillary canines, but the 3D segmentation process, even if applied only for the crown scans, entails a prolonged time consumption.

Since most authors confirmed that central incisors show a stronger correlation between aging and pulp chamber volume decrease than premolars and canines,[38-40,42] Pinchi et al. developed and validated a novel method for adult age estimation based on a geometric approximation system of dental volumes using CBCT scans.[43] Measurements were conducted on CBCT (Scanora 3D – Soredex) sagittal and axial scans of upper left central incisors. For the correlation between the volumes ratio and end age, a linear regression formula and a Bayesian statistical distribution method have been developed. [43-44] They concluded that the proposed approach remarkably reduced the operating time in comparison to the other more complex techniques based on the segmentation model.

The easy approach, the accuracy of the age predictions, and the evidence of no statistically significant differences between sexes in agreement with previous research, [22, 39-40] led choosing for this study the method of adult dental age estimation developed by Pinchi et al. [43-44] This method was applied to assess the age at death of subjects found in two different Italian necropolis (in Florence under the Uffizi Museum and in Sardinia, in Mont'e Prama) to investigate the reliability of the age estimates in ancient population of archeological interest.

In 2012, the forensic odontology group of the University of Florence was requested for a joint-field research with the Authority for Archaeological Heritage of the Tuscany Region (Italy) to assess the age at death of human remains found in two different necropoleis, in Mont'e Prama (Sardinia) and then in Florence (Tuscany).

The two archeological areas of interest differ in several aspects: geographical site, historical era, graves setting, bones preservation. The necropolis of Mont'e Prama is located near Cabras (Sardinia) and the burial site consists of a total of 33 tombs cylindrical shaped dug in the ground and covered with chalky sandstone slabs. The skeletons of young adult males were buried in sitting or kneeling positions and the probable long time of exposure to the outside led to an extreme fragmentation of skeletal and skull bones (Fig. 1).

Figure 1. Human remains found inside a tomb of Mont'e Prama: Official images from "Mont'e Prama Foundation" website.

Fragments of stone statues, named "The Giants of Mont'e Prama", covered the graves and have been dated to the X-IX century B.C according to the radiocarbon analysis. Genetic analyses were conducted especially on available teeth. The historical period and the results obtained from the genetic and morphological analyses on remains suggest that the necropolis of Monte Prama might be the burial space reserved for a family group of young adults dominant in the Nuraghic society of the First Iron Age or a group of male warriors or athletes. Conversely, the necropolis of Florence is located near the Arno River. According to the radiocarbon dating of some groups of coins found alongside some skeletons, it probably dates from the V-VI century A.D. Seventy-five skeletons of adults and children (both males and females) were found concentrated in a small area, buried aligned in groups of three to eleven individuals in an alternate head-to-toe manner with children's skeletons deposited in the free spaces between the adults' bones (Fig. 2). The small area of the necropolis settled along the riverside, the disorderly position of the skulls and upper limbs, and the absence of signs of fatal wounds or starvation, have been interpreted by the archeologists as the result of hasty burial due to a special emergency condition that led to the death of many people in a short time-lapse and in a critical condition of lack of space outside the town: a deadly epidemic plague in the town (Black Death) or an epidemic of salmonella during a very hot summer.

Fragments of stone statues representing 25 young male warriors and 13 models of "*nuraghes*" covered the graves. The statues were named "The Giants of Mont'e Prama" and have been dated to the X-IX century B.C according to the radiocarbon

analysis. Genetics analyses were conducted especially on available teeth.
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Figure 2. Human remains found inside a tomb of Florence lying in a disorderly position of the skulls and upper limbs head-to-toe: image from "Toscanaoggi" website.

MATERIALS AND METHODS

The about 108 skeletal remains recovered from Mont'e Prama and Florence underwent anthropological and dental age estimation analyses.
No inclusion criteria were applied for the anthropological age estimation sample collection. The age estimation methods were selected according to the available remains.
Inclusion criteria for the dental age estimation sample:

- The availability of intact and sound left central incisors;

Exclusion criteria for the dental age estimation sample:

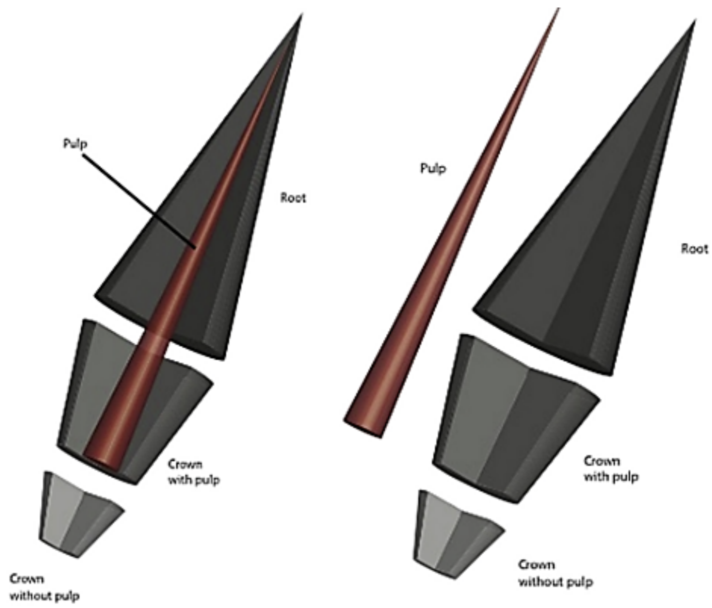
- All the skeletal remains missing left central incisors were excluded from the analyses.

For the anthropological age estimation, morphological methods based on the assessment of the ecto/endo cranial suture closure, parietal thinning, pubic symphysis face and auricular surface of the ilium metamorphosis, development of the sternal rib ends, osteoarthritis, radiographic evidence of the bone loss of clavicle and proximal femur, tooth wear, and bone histology features, [22,45-51] were applied according to the available remains obtaining wide classification ranges from *sub-adults* (younger than 18 year sold), *young adults* (from 18 to 20-25 years old), and *adults* (older than 30 years). In particular, the Lovejoy et al. method based on tooth wear [22] has been applied when jaws were available. Tooth wear is classified into 9 stages for all maxillary dental elements and 10 for mandibular teeth (including third molars). Estimate inaccuracies result in line with those from other anthropological methods based on pubis, ilium, femur, and sutures (varying from 7,4 up to 12,2 years), thus suggesting the applicability in case of teeth availability but providing small ranges of age estimate (4–10 years) never overlapped (i.e., 25–29, 30–34, 30-40 etc.). [22]
For the dental age estimation, the Pinchi et al. method of geometrical approximation of dental volumes [43] and the Bayesian statistical distribution [44] were applied to the available left upper central incisors.

A CBCT of the entire skulls, jaws or single teeth (Fig. 3) was taken and two experienced forensic odontologists performed the high and area measurements on sagittal and axial CBCT scans to calculate dental volumes according to Pinchi et al. methods [43-44].
This method calculates the different dental volumes (dental hard tissues, total volume of the tooth, and pulp volume) throughout a geometrical approximation of the different parts of the tooth (crown, roots, and pulp chamber).
The root and the pulp chamber are assimilated into an elliptical based cone; whilst the crown to an elliptical based truncated cone (Fig. 4).
The ratio between the pulp volume and the hard tissues (dental) volume is then measured according to the following equation: [43]*PHr* [ratio between the pulp volume and the hard tissues (dental) volume] = *V pulp/V ht* with the *V ht* [volume of dental hard tissues] = *Vtot* [total volume of the tooth] − *V pulp* [volume of the pulp].

Figure 3. CBCT sagittal and axial scans and 3D reconstructions of skulls, jaws, and teeth from CBCT acquisitions.

Figure 4. Geometrical approximation of the tooth. The root and the pulp have been assimilated into elliptical-based cones and the crown into an elliptical-based truncated cone [43]



Statistical analysis

The statistical distribution of age and errors followed the Bayesian approach proposed by Sironi et al. [44] The estimate of the age of each subject was obtained through the application of the following Bayes' theorem:

$$p(a \mid e,\theta) = \frac{p(e \mid a,\theta) \times p(a)}{\int p(e \mid a,\theta) \times p(a)da}$$

where *e* is the natural logarithm of the PHr; *p(a | e,θ)* is the linear relationship between the evidence and the age of the subjects with an uncertainty Normally distributed; θ represents the parameters of the Bayesian model estimated on a dataset of contemporary subjects collected from two private radiological studies, in Northern and Central Italy; *p(a)* is the "*a priori*" probability of the age of each subject individually attributed in a range of a *maximum* and a *minimum* value on the basis of available information. A Standard Deviation (SD) for each evaluation was calculated as the Mean Squared Error of the discrepancy between the age estimated with the Pinchi et al. method[43] and the age assessment obtained with the anthropological methods, according to the Bayesian model proposed by Sironi et al. [44]

The *maximum* and *minimum* setting of the "*a priori*" ranges was attributed differently for the two necropoleis groups, depending on the anthropological evaluations and historical information available. For the sample of Mont'e Prama, a *uniform priors* model was assessed considering the group homogeneity, thus the same *prior ranges* were imposed for all the subjects. The minimum age was set according to the previous anthropological estimates and complete dentition where possible; the maximum age was defined according to the average surviving age at that time. Due to the heterogeneity of the Florentine sample, a double validation of the age assessment was conducted, applying *uniform priors* in the first evaluation and *informed priors* for a second evaluation (intended as personalized prior ranges according to the specific morphological or physical characteristics of the remains). The *uniform priors* were established according to the presence of permanent dentition, since the jaws were complete and available for all samples, and the maximum surviving age at the time. The *informed priors* were obtained for each subject according to personal dental data (tooth maturation and wear assessment, presence and formation of wisdom teeth) and on the lifestyle of the Florentine civilization in the respective historical period defined by the archaeologists.

The results obtained from the two prior models on the Florentine sample were then compared to appreciate if the informed prior could significantly improve the Bayesian evaluation in heterogeneous ancient populations.

RESULTS

Only 15 skeletons from Mont'e Prama Necropolis and 22 from the Uffizi Necropolis met the inclusion criteria for the dental age estimation.

The complete dental procedure, including the CBCT exposure, the image reconstruction, the pre-processing analyses (drawing the boundary of the images manually), and the post-measurements, required less than 15 minutes for each tooth.

For the sample of Mont'e Prama, the "*a priori*" minimum age was set at 12 years old, according to the previous anthropological estimates which defined all the subjects as *young adults* all in permanent dentition (including second molars). The maximum age was set at 35 and 40 years old, respectively, since the average surviving age at that time [Late Bronze Age to the Early Iron Age] was about 35/40 years old.

Table 1 shows the results of the Mont'e Prama sample obtained with the Pinchi et al. method [43] and the estimates obtained with the morphological methods. [45-51] The SD (standard deviation) was calculated between the Pinchi et al. and anthropological assessments. The age estimation with the "*a priori*" setting up to a maximum of 35 year varies from 18.4 up to 28.7 years with a minimum error between dental and anthropological analyses of 5,15, and a maximum error of 6,14 years. The age estimation with the "*a priori*" setting up to a maximum of 40 year varies from 18.71 up to 31,41 years with a maximum error of 7,14 years.

Table 1. Results of the sample from Mont'e Prama Necropolis with the "a priori" settings between the age range 12-35 and 12-40 years.

Sample ID	Dental Volumes Ratio	Prior U (12; 35)		Prior U (12;40)		Anthropological estimates
		Estimated dental age	SD	Estimated dental age	SD	
1	0,056	23,63	6,11	25,03	7,13	Young adult (20 years)
2	0,075	19,57	5,54	19,98	6,09	Young adult (20 years)
3	0,082	18,68	5,24	18,96	5,66	Young adult (20 years)
4	0,069	20,56	5,80	21,15	6,48	Young adult (20 years)
5	0,045	25,57	**6,14**	27,75	7,12	Young adult (20 years)
6	0,084	**18,46**	**5,15**	**18,71**	5,54	Young adult (20 years)
7	0,053	24,69	6,06	26,47	**7,14**	Young adult (20 years)
8	0,041	**28,07**	5,32	**31,41**	6,29	Young adult (20 years)
9	0,061	22,21	6,05	23,19	6,93	Young adult (20 years)
10	0,062	22,02	6,03	22,95	6,89	Young adult (20 years)
11	0,077	19,23	5,43	19,60	5,94	Young adult (20 years)
12	0,057	23,49	6,11	24,84	7,11	Young adult (20 years)
13	0,048	26,02	5,87	28,34	6,99	Young adult (20 years)
14	0,056	23,56	6,11	24,95	7,13	Young adult (20 years)
15	0,082	18,71	5,25	18,99	5,67	Young adult (20 years)

According to the information from the life conditions in the town of Florence in the Roman Era [V-VI century A.D.], for the *"a priori"* settings a minimum age of 12 years was established since all the subjects were in permanent dentition, and a maximum age of 70 years, since the unknown variability of the population examined (*uniform priors*). The anthropological estimates varied from sub-adults up to adults, and for all the sample the Lovejoy et al. method based on tooth wear [22] could be applied, due to the presence of complete jaws. The SD (standard deviation) was calculated between the Pinchi et al. and the tooth wear Lovejoy et al. assessments.

Table 2 shows the results of the Florentine sample. The dental age estimation varies from 12.6 up to 49.31 years with a minimum error of 0.99 and a maximum of 8,8 years characterizing a heterogeneous sample.

A second phase of analysis was conducted in accordance with further information acquired from the available dental data, tooth wear assessment,[22] and on the lifestyle of the Florentine civilization in that specific historical period defined by the archaeologists. It was possible to reduce the range of *"a priori"* variability of the age of the sample, setting the minimum limit at 15, 18, and 23 years respectively depending on the characteristics of the individual subject (complete permanent dentition, complete second molars, or advanced formation of the root of wisdom teeth), and the maximum limit at 50 years (*informed priors*). All subjects with wisdom teeth with complete apex were evaluated with a *minimum prior* greater than 23 years, while those with wisdom teeth still in formation were considered a minimum of 15 or 18 years. The maximum age was placed at 50 years considering the more specific average survival information.

In Table 3, the results obtained in the Florentine sample with the *informed priors* set according to the individual characteristics at 15, 18, and 23 years up to 50 years. The age estimation varies from 15.88 up to 43.37 years with a minimum error of 1 year up to a maximum error of 7,85 years confirming the heterogeneity of the sample of Florence.

Table 2. Results of the sample from the Uffizi Necropolis with the *" a priori"* settings between the age range 12-70 years.

Sample ID	Dental Volumes Ratio	Estimated dental age	SD	Lovejoy estimates (dental wear)	Anthropological estimates
1	0,037	44,48	7,62	40-50	Adult
2	0,052	27,71	**8,8**	24-30	Young adult
3	0,094	12,60	5,41	18-24	Young adult
4	0,069	14,89	7,29	16-20	Young adult
5	0,078	12,89	6,45	16-20	NA*
6	0,088	**12,62**	5,74	16-20	Young adult
7	0,087	12,61	5,81	16-20	Sub-adult
8	0,028	**49,31**	5,78	20-24	Young adult
9	0,099	24,89	4,52	18-22	Young adult
10	0,103	22,94	4,48	20-24	Adult
11	0,060	21,72	8,27	16-20	Young adult
12	0,053	27,79	8,79	24-30	NA*
13	0,068	15,50	7,4	24-30	Adult
14	0,063	18,20	7,91	16-20	Young adult
15	0,046	34,45	8,76	20-24	Young adult
16	0,087	23,75	5,11	20-24	Young adult
17	0,043	37,21	8,55	24-30	Young adult
18	0,094	15,87	**0,99**	12-18	Sub-adult
19	0,057	23,99	8,55	24-30	Adult
20	0,074	13,06	6,82	NA*	Adult
21	0,076	12,94	6,59	12-18	Sub-adult
22	0,056	24,31	8,56	24-30	Adult

*NA: not available

Table 3. Results of the sample from the Uffizi Necropolis with the *"informed priors"* settings between the age range 15-50 years.

Sample ID	Dental Volumes Ratio	Prior I minimum	Prior I maximum	Estimated dental age (SD)	CI 95%	Lovejoy estimates (dental wear)	Anthropological estimates
1	0.037	23	50	39.74 (6.65)	25.51 – 49.48	40-50	Adult
2	0.052	23	50	33.22 (6.66)	23.51 – 47.5	24-30	Young adult
3	0.094	18	50	23.32 (4.85)	18.15 – 36.11	18-24	Young adult
4	0.069	18	40	25.22 (5.33)	18.27 – 37.51	16-20	Young adult
5	0.078	18	40	24.20 (4.96)	18.2 – 36.46	16-20	NA*

6	0.088	18	40	23.46 (4.63)	18.16 – 35.37	16-20	Young adult
7	0.087	15	30	20.10 (3.83)	15.17 – 28.71	16-20	Sub-adult
8	0.028	23	50	**43.37** (5.45)	29.54 – 49.78	20-24	Young adult
9	0.099	20	50	24.90 (4.53)	20.14 – 36.93	18-22	Young adult
10	0.103	18	50	22.94 (4.58)	18.14 – 35.07	20-24	Adult
11	0.060	18	40	26.89 (5.73)	18.42 – 38.56	16-20	Young adult
12	0.053	23	50	33.04 (6.62)	23.49 – 47.28	24-30	NA*
13	0.068	23	50	29.74 (5.52)	23.22 – 43.71	24-30	Adult
14	0.063	15	30	21.99 (4.17)	15.36 – 29.46	16-20	Young adult
15	0.046	18	40	30.61 (5.79)	19.26 – 39.51	20-24	Young adult
16	0.087	18	50	23.76 (5.13)	18.17 – 37.2	20-24	Young adult
17	0.043	18	50	35.66 **(7.85)**	20.26 – 48.88	24-30	Young adult
18	0.094	15	50	**15.88 (1.00)**	13.92 – 17.83	12-18	Sub-adult
19	0.057	23	50	31.88 (6.33)	23.37 – 46.45	24-30	Adult
20	0.074	23	50	29.08 (5.19)	23.19 – 42.54	24-30	Adult
21	0.076	15	30	20.74 (4.00)	15.22 – 29.06	12-18	Sub-adult
22	0.056	23	50	31.96 (6.36)	23.38 – 46.56	24-30	Adult

*NA: not available

DISCUSSION

Age estimation is a critical aspect of analyzing human skeletal remains, both in the context of forensic identification (whether involving individual or mass deaths) and in anthropological and archaeological research, particularly for paleodemographic studies. [1-18] Given that ancient skeletal remains often provide limited evidence and must be preserved for archival purposes, it is essential to develop and apply conservative, reliable methods for age estimation, particularly those focusing on teeth.[20]

This research aimed at verifying the feasibility and repeatability of the adult age estimation method developed on living people by Pinchi et al. [43-44] for estimating the age at the death of subjects from ancient populations found in two different Italian necropolis of archeological interest. The method is extremely conservative since it is based on CBCT scans obtainable from Post-mortem entire skulls, jaws, or teeth. Furthermore, the entire procedure requires about 15 minutes for each tooth analysed.

The study confirms the significant correlation between the reduction of the pulp chamber volume due to the progressive deposition of secondary dentin during the life of an adult subject and the adult age even in ancient populations. The geometrical approximation of dental volumes measured on CBCT

can be suggested as a reliable tool for estimating age in both modern and ancient populations, since it consists of a non-invasive/destructive three-dimensional radiographic technique and the correlation with the adult age is independent of sex. [22, 39-40, 43-44] These features represent clear advantages in estimating the age of ancient populations or archaeological/anthropological interest since from human/skeletal remains it is often impossible to obtain all the information needed to construct the entire biological profile without using invasive methods (genetics) and therefore without sacrificing tissue.

A graduate and progressive scientific methodology combining the anthropological and dental age estimation was conducted to validate the Pinchi et al. methods [43-44] in the ancient populations. The inaccuracy in the dental age estimation (SD compared to morphological evaluations [22, 45-51]) varies from 5,15 up to 6,14 years in the sample of Mont'e Prama (Table 1), and from 1 up to 7,85 years in the sample of the

Necropolis of Florence (Table 3) with a mode of error of 4-5,5 in almost all age cohorts (from sub-adults 16-18 years old, up to adults older than 40 years). Some authors [52] underlined that the Lovejoy methods [22, 45-50] for age estimation, such as the other morphological assessments usually practiced by anthropologists [51], consistently perform low accuracy for all age cohorts (from 21 up to 79 years old) providing small ranges of age estimate (5–10 years) never overlapped (i.e., 25–29, 30–34, etc.). This limit could lead to a higher risk of a systematic bias excluding the real age of the human remains. Otherwise, the prior selection of possible age ranges could strongly improve Bayesian methods to provide more accurate age estimates within the established age cohorts. The Pinchi et al. methods [43-44] demonstrated to perform with high accuracy for all the age cohorts of ancient sub-adults and adults confirming the skeletal age estimation with a narrower inaccuracy range, without a systematic under or overestimation, and with higher reliability offering age estimates ranging between 10-12 years and CI 95% of error between 10 and 20 years (Table 3).

Furthermore, the analyses conducted with the Bayesian approach [44] indicate that outcomes in terms of accuracy might be considerably improved by integrating *prior* knowledge in the estimation procedure. For example, case 3 out of the Florentine sample gained an inaccurate estimate in Tab. 2 according to the tooth volume ratio evaluated within wider *prior* ranges, and an age younger than 18 years old was attributed (12,60 years old). After the application of informed priors, obtained from the estimates based on tooth wear and anthropological methods (Tab. 3, 18-24 years old), a minimum age of 18 years was set and the Pinchi et al. method evaluated 23.32 years old, in line with anthropological results. Comparing the results obtained on the Florentine sample in Tab. 2 (using a *uniform priors* with a wider range of variability) with the results obtained in the second analysis in Tab. 3 (using *informed priors* with narrower and personalized range obtained with the further archaeological/anthropological and dental knowledge) there is a significative reduction in the maximum average error (from 8.8 years in Tab.2 to 7.85 years in Tab. 3) which however is concentrated for the age cohorts between 30 and 50 years (from 5.79 up to 7.85 years). These findings are in line with Yang et al. [53] which obtained a square root of mean square error of 8.3 years applying CBCT measurements on the pulp chamber/tooth volume ratio on incisors and canines of a modern population mostly aged from 20 to 50 years. Similar results (mean absolute errors of 8.54 years) were gained by Jagannathan et al. [54] on a CBCT canines sample and by Ge et al. [55] demonstrated the highest variability of pulp chamber/tooth volume ratio between the age span from 25 to 40 years.

Whilst, the results of this study highlight an opposite trend compared to the Pinchi et al. [43] applied for the same age cohorts in living people, in which the highest accuracy in age prediction was obtained with residual errors of 0.71, 2.88, and 5.86 years. Similarly to Aboshi et al., [56] their results are in line with what is known regarding the physiology of secondary dentin deposition, which presents a reduced speed in young adults (< 30 years) who have just completed the dental maturation, then increases considerably in the average adult up to a severe slowing down in adults >60 years.

These significant dissimilarities could be attributed to the different population analysed (modern and ancient) and the variability in the rate of tooth wear of the upper central incisors to which archaeologists report to be more exposed in correlation with lifestyles, habits, and with the physical properties of the consumed food and their preparation processes in ancient eras. [57] Therefore, the presence of major tooth wear could influence the dental age estimation based on the geometrical approximation of central incisor volumes resulting in an over-estimation in the adult age cohorts that has to be considered in studying archaeological populations, e.g. especially farmers. On the other hand, the estimates obtained using the *informed priors* of the Pinchi et al. method [43-44] led to a reduction of the effects of dental wear on age assessment accuracy. Case 8 out of the Florentine sample, reveals a decrease in age evaluation in Tab. 3 which considers the results by Lovejoy et al.[22] suggesting a minor tooth wear of the whole jaws than of the solely upper lateral incisor.

Since the high heterogeneity of the Florentine sample and the limited information available, our results can be considered in line with those obtained in previous studies, and even more accurate than those obtained by De Angelis et al. [58] on canines. They calculate a prediction interval of around ±30 years with 95 % confidence for both males and females and a prevision interval of ±12 years in the female group and ±14 years for males at 60 % confidence interval. Similarly, Marroquin Penaloza et al. [59] applied the Kvaal method on CBCTs of a modern population with a median age of 31 years (with different errors for diverse combinations of measurements from ±10.58 years up to ±12.84 years). According to Sironi et al., [44] in the *informed Bayesian* approach the real risk is the expert's initial belief which could sensitively differ from the real age of the individual examined, leading to incorrect estimates. However, in the case of the Florentine population, a multi-trait approach was used in the *a priori* definition of each subject. The different information collected from the other dental elements present in the subjects' oral cavity, considering the complete permanent dentition, the complete formation of second molars, the advanced formation of the wisdom tooth's root until stage D of the Demirjian method, [60] and the maximum survival age of the historical period. The reduction of the standard error in almost all the estimates from Table 2 (*uniform priors*) to Table 3 (*informed priors*) confirms the better reliability of the results obtainable with narrower *a priori* age ranges. These results demonstrate the variability of the age sample suggesting to the archeologists that they were different family groups buried all together in a small area near to the center of the town.

On the contrary, the Mont'e Prama group demonstrates excellent accuracy of the measurement method applied with uniform Bayesian statistics (Tab. 1). The sample resulted extremely homogeneous so the error distribution was similar for all age cohorts and the accuracy obtained was even higher than Florentine group. According to the results obtained with the *prior* interval of 12-35 years, it seems to better identify the real age of the entire sample with reduced deviation from skeletal age estimation (Table 1). The analysis shows the absence of infants and elderly people, the presence of 5 subjects aged between 18-19 and the prevalence of "young adults" aged between 20 -30 years, all of them males. The age and the sex of the buried bodies led archeologists to define the deceased population of Mont'e Prama as a probable group of warriors or athletes.

The main limitation of the Pinchi et al. methods [43-44] is the availability of the left upper central incisors. Of the entire sample discovered both in Florence and Sardinia, only 37 subjects presented the required characteristics (22 from the Uffizi Necropolis and 15 from the Mont'e Prama Necropolis) and then could be estimated. Despite the limited availability of information provided by the recovered remains, approximately 30% of the total cases (about 108) could be estimated from the only use of dental technique.

CONCLUSION

The pulp chamber narrowing, measured using tooth geometrical approximation on CBCT scans, demonstrates to be a reliable and conservative tool for estimating age in adult ancient populations of archaeological interest, offering an accuracy comparable to other methods that are invasive or destructive.

The *informed priors* model compared to the *uniform* one in a heterogeneous ancient population reveals a better accuracy in age estimation, but the *prior* setting needs reliable personalized information (e.g. dental maturity, etc).

The inaccuracy of dental age estimation, compared with skeletal age estimation, typically ranges from 1 to 8 years, with no systematic overestimation or underestimation, but in some cases could be influenced by significant tooth wear according to specific working or dietary habits of ancient populations.

Given the differing compositions of the two analyzed samples, the resulting age estimates are endowed with palaeodemographic value for archaeologists and anthropologists. The Mont'e Prama sample was identified as a homogenous group of males aged around 20–30 years, suggesting they were warriors, soldiers, or athletes. In contrast, the population of Florence was more heterogeneous and probably composed of individuals from different families buried together in a small area due to a special social emergency, likely associated with a plague epidemic in the city.

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