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Comparison of the validity of three dental methods for the estimation of age at death

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Abstract

The aim of the present study was to compare the accuracy, precision, and bias of two macroscopic and one histological age at death estimation methods on human teeth. The sample was comprised of 67 permanent teeth, obtained from 37 individuals aged 20–91 years. Age was predicted according to the methods proposed by Lamendin et al. (LAM), Bang and Ramm (BR), and the quantification of tooth cementum annulations (TCA).

TCA was found to be most accurate in all age groups. Its mean absolute error of the estimated age was about half as high as the mean absolute error for both LAM and BR. BR achieved approximately the same mean absolute error as TCA for old adults only.

LAM displayed the highest precision in the young and the old age group whereas TCA was more precise in the middle age group. TCA was found to be the most precise method when the precision was calculated for all ages.

Considering the bias, all methods displayed a tendency to overestimate age in young and to underestimate it in old specimens. The exception to this rule was TCA, which provided unbiased estimates for young adults.

The higher accuracy and precision recommends favouring TCA over LAM and BR, provided that the required know-how and equipment are available.

Keywords: Forensic odontology; Human teeth; Age at death estimation; Identification; Comparison of methods

1. Introduction

Age at death estimation of human remains constitutes an important contribution to the identification process of missing people, and also provides valuable data in the paleodemographical analysis of historical skeletal remains. The dental hard tissues belong to the most durable substances produced by the human body and show the best resistance against postmortem alterations caused by humidity, high temperature, microbial activities, and mechanical forces.

Moreover, several techniques for dental age estimation are reported to be more accurate than age predictions based on other somatic traits such as the closure of the cranial sutures [1] or the decline in the architecture of the trabeculae of the femoral head [2].

Dental age estimation methods are either based on the well-ordered cascade of changes that occur during the formation and eruption of teeth or they rely on continuous processes that alter and diminish the quality of dental tissues even when individual growth is completed. Nevertheless, the validity of age at death

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estimations in adults using these variables still remains to be evaluated in greater detail and in controlled studies.

Dentin and cementum are hard substances that are, contrary to enamel, continuously synthesized and maintained throughout all stages of a person's adult life. These gradual and lifelong changes make dentin and root cementum ideal substrates for the study of aging processes and it has been demonstrated that they can provide valuable information for the estimation of age at death [3–5].

Age-related transparency of the tooth root is a physiologic trait which appears after the age of 20 and is a result of the deposition of a densely packed matrix with hydroxyapatite crystals within the dentinal tubules. This leads to an equalization of the refractive index between the intra-tubular and inter-tubular dentin, which causes an increased transparency of the affected dentinal regions [6,7]. The transparency progresses from the root apex towards the cervical region and can be visualized when the tooth is placed on a light box [6]. Lamendin et al. [6] used the tooth root transparency for age estimation in combination with gingival regression (periodontosis), for which an additional measurement of the tooth length is necessary.

Gingival regression, another age-dependent feature, is a multifactorial process, which was introduced for age estimation by Gustafson [8]. However, pathologic factors may play a major role in the occurrence of gingival regression and it therefore constitutes one of the criteria which have to be evaluated critically. Lamendin et al. [6] established a regression formula, which is suitable for both sexes and ought to be applicable to all types of single rooted teeth. Thus, Lamendin et al.'s method (LAM) is easy to apply and uses only one single rooted tooth that does not require further preparation or specialized technical equipment.

Bang and Ramm (BR) [3] also studied the phenomenon of transparent dentin, but in a more sophisticated way. They presented regression equations for sectioned and unsectioned teeth considering tooth type and the length of root transparency that included multi-rooted teeth. The authors proposed formulae dependent on the length of the transparency as the sole morphological predictor for the estimated age [3].

Thirdly, the evaluation of tooth cementum annulations (TCA) was chosen to compare a popular histological method with the two macroscopic procedures. Cementum is a hard tissue with mineralized organic matrix, which does not undergo continuous remodeling. It is formed as primary acellular cementum and secondary cellular cementum that is arranged in layers (lines) surrounding the human tooth root dentin. This layered appearance is due to structural differences in the mineral phase, an optical phenomenon that is possibly related to altered mineral crystal orientations [9] and reflects a cyclic annual formation pattern. One pair of alternating light and dark lines should therefore correspond to 1 year in an individual's life. If the count of cementum annulations is added to the tooth-specific eruption age, the result is an estimate of the chronological age. A possible explanation for the layered organization of human cementum is the fact that growth hormone affects cementogenesis [10–12] which could be influenced by environmental factors such as the seasonal change [13–15].

In 1950, Gustafson [8] was the first to report the use of cementum layer width in human age estimation; 8 years later Zander and Hurzeler [16] established the linear relationship between an individual's chronological age and the continuous growth of cementum. In the following decades, a number of scientists used the quantification of tooth cementum annulations to estimate the age of a variety of wild animals although the underlying mechanism of its layered nature is not fully understood. Inhomogenous results were obtained from the TCA of human teeth, the observations ranged from an undetectable relationship between the number of incremental lines and age [17–19] to a well-correlated connection [5,20,21]. However, some questions regarding the precision and accuracy of TCA analysis in comparison with other dental age estimation methods are still unanswered. Some authors decided to choose the most promising pictures, to exclude teeth with irregularities from TCA analysis and to increase the number of tooth sections if required. This might be a critical point with regards to the comparability of different studies as well as making it more difficult for the scientist to carry through the adequate procedure.

Table 1
Number of teeth subdivided by tooth type and sex

	Tooth type						Total
	Central incisor	Lateral incisor	Canine	First premolar	Second premolar	First molar	
Upper jaw							
Sex							
Male	0	3	2	5	3	0	13
Female	2	1	0	0	2	0	5
Total	2	4	2	5	5	0	18
Lower jaw							
Sex							
Male	6	8	3	14	6	1	38
Female	1	1	6	3	0	0	11
Total	7	9	9	17	6	1	49

In this paper, we present a study with the goal of evaluating the potential performance of three methods in dental age at death estimation of human remains and to assess their objectivity and reproducibility.

2. Material and methods

The sample consisted of 67 permanent teeth from 37 individuals of known age and sex. The age ranged from 20 to 91 years with a mean of 50.7 years old. A detailed distribution of the number of teeth subdivided by tooth type and sex is shown in Table 1. All teeth chosen for the analysis had to be free of any manifestations of traumatic insults, carious lesions and visible signs of resorption in the region of the tooth root.

Of the teeth 37 were obtained from individuals who had donated their bodies to the Centre for Anatomy and Cell Biology, Vienna, for science and medical education. These specimens were fixed in buffered 4% formalin solution after removing residual soft tissue.

Another 30 teeth were collected from the anatomical Weisbach Collection, which is held by the Natural History Museum, Vienna. This collection offers the chance to study skeletal material of known age and sex, documentation in cases of pathologic findings makes it possible to avoid such cases. After the macroscopic assessments, a silicon replica of each specimen was constructed. The tooth crown was cut off and returned with the replica to the collection in order to fulfill curatorial obligations and to provide morphological information. The tooth root was subjected to the preparation of thin-ground sections.

As the skeletal material was air-dried, these teeth did not require a fixation procedure.

Table 2 lists the number of anatomical and skeletal specimens subgrouped by sex. The number of teeth in each age group is shown in Fig. 1.

2.1. LAM

Measurements were taken according to the method outlined in Lamendin et al. [6]. This included the assessment of the root height using a pair of sliding callipers (CD-15DC, Mitutoyo, Japan), a millimetre ruler, and a desktop lamp. The root height is defined according to Lamendin et al. as "the distance between the apex of the root and the cementoenamel junction", measured on the labial surface of the tooth.

Periodontosis (gingival regression), which is described as "the maximum distance between the cementoenamel junction and the line of soft tissue attachment" [6], was measured at the labial surface of the tooth.

The third dental feature, the transparency of the root, is defined as follows "from the apex of the root the maximum height of T is measured on the labial surface of the tooth" [6]. Therefore, the transparency was assessed on the labial surface with the help of a light box (slim line 4 cm, Planistar, Himmelstadt, Germany). The estimated age was obtained using the equation $A = 0.18P + 0.42T + 25.53$ as proposed by Lamendin et al. [6], where A indicates the estimated age, P periodontosis (periodontosis height × 100/root height) and T transparency (transparency height × 100/root height). Each parameter was measured and recorded three times. The mean of the three measurements was taken for further analysis.

For the assessment of intra-observer and inter-observer variability, 10 teeth were chosen at random. All measurements were taken under blinded conditions and at different occasions to avoid potential bias.

Table 2
Number of anatomical and skeletal specimens subgrouped by sex

	Sex		Total
	Male	Female	
Anatomical	23	14	37
Skeletal	28	2	30
Total	51	16	67

2.2. BR

Measurements were taken according to the method presented by Bang and Ramm [3] by using a sliding calliper (CD-15DC, Mitutoyo, Japan), a desktop lamp and a light box (slim line 4 cm, Planistar, Himmelstadt, Germany). The total length of the root (RL) was measured "bucally and in the midline from the cementoenamel junction to the apex". The transparent root dentin was "measured from the apex of the root in coronal direction to the borderline between transparent and opaque dentin". Here a minimal (TL1) and a maximal (TL2) value of the root transparency were recorded, in cases of a horizontal line TL1 = TL2. The TM (the mean of TL1 and TL2) value was chosen as the variable for estimation of the chronological age. According to Bang and Ramm TM was calculated (TL1 + TL2)/2 [3]. The estimated age was assessed using the formula $A = B_0 + B_1 \times X + B_2 \times X^2$ (transparent length less than or equal to 9.0 mm) and the formula $A = B_0 + B_1 \times X$ (transparent length more than 9.0 mm) employing the coefficients for intact roots as presented in the original paper [3]. For each measurement, triple-recordings were performed. The mean value of the three measurements was subjected to evaluation.

For the assessment of intra-observer and inter-observer variability, 10 teeth were chosen randomly. All measurements were taken under blinded conditions and at different occasions to avoid potential bias.

2.3. TCA

The teeth were subjected to the preparation of undecalcified thin-ground sections as described elsewhere [22,23]. Briefly, the extracted teeth were fixed in buffered 4% formalin solution, dehydrated in ascending grades of alcohol and embedded in light curing resin (Technovit 7200 VLC with BPO, Kulzer, Germany). Further processing was carried out with the Exakt Cutting and Grinding Equipment (Exakt Apparatebau, Norderstedt, Germany). The specimens were cut perpendicular to the root axis at the middle third of the root and three subsequent thin-ground sections of 90–100 μm were prepared. For the analysis of tooth cementum annulations, three digital photographs (Nikon Coolpix 990, Nikon, Tokyo, Japan) of each section were taken with high contrast. The digital camera was mounted on a Nikon Eclipse 50i microscope (Nikon, Tokyo, Japan), resulting in pictures in which 1 mm measured 7220 pixels (1 pixel = 0.139 μm). The pictures showed the layered appearance of cementum with alternating light and dark lines. The images were saved for examination and counting. The tooth cementum annulations (only the dark lines) were interactively highlighted and counted using Adobe Photoshop® (Adobe, San Jose CA, USA). This process resulted in nine counts per tooth (three sections, three photographs per section) which were conducted by one observer. To obtain the age at death estimate, the mean of the line counts was added to the sex-specific eruption age of each tooth [24,25]. For the assessment

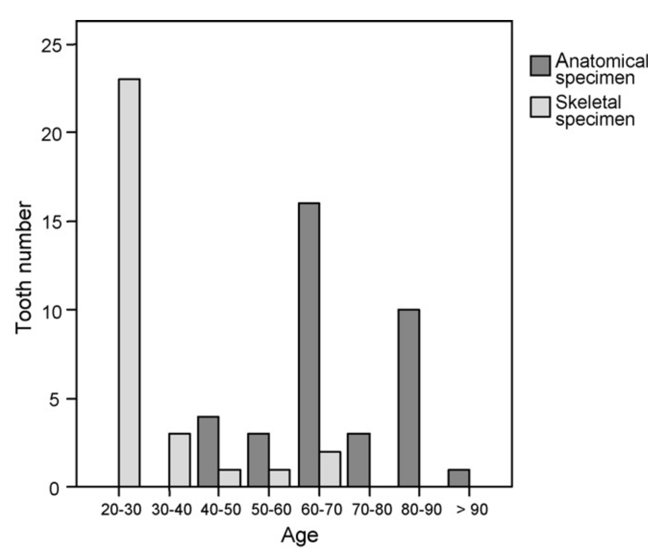

Fig. 1. Tooth number per age group for anatomical and skeletal specimens.

of intra-observer and inter-observer variability, 50 photographs were chosen at random. The photographs were recounted under blinded conditions and at different occasions to avoid potential bias.

2.4. Statistical analysis

For the assessment of inter-observer and intra-observer variability, the intraclass correlation coefficient (ICC) was used.

The three methods were compared with respect to bias, precision and accuracy. Bias was evaluated numerically by computing the mean error [ME = $\sum$(estimated age − true age)/n], precision was measured as the standard deviation and accuracy was defined as the mean absolute error [MAE = $\sum$Abs(estimated age − true age)/n]. For definitions and mathematical formulations see Walther and Moore [26].

In addition to the numerical comparisons of the three methods, residuals (estimated age − real age) were plotted against the real age. Aykroyd et al. [27] show, that because of mathematical reasons the slope of the regression of residuals on true age is not zero, but $R^2 - 1$ (R is Pearson's correlation coefficient of the original regression). This leads to an overestimation of younger individuals and an underestimation of older individuals. In the case of LAM, R^2 was reported to be 0.33, so the slope of the regression of residuals on age should be approximately −0.66. BR provides several polynomial regression equations, for each tooth type separately. Therefore, the slope of the residuals was only roughly estimated by computation of the mean of several correlation indices (0.767), resulting in a slope of −0,412. These slopes were used to plot the regression lines in Fig. 2 to show the extent of this mathematically explainable error.

The statistical comparison of the predictive power of the measured variables (transparency according to LAM, transparency according to BR, periodontosis, cementum layer count) was performed by fitting several linear regression models with age as the dependent variable. A description of the test for comparing the models can be found by Faraway [28].

Another regression analysis was performed to evaluate potential differences between Lamendin's original equation and the present sample. Therefore, a multiple regression model was employed for a subsequent comparison of the resulting regression coefficients.

All statistical calculations were carried out by using the statistical software R 2.5.0 [29].

3. Results

3.1. Intra-observer and inter-observer variability of LAM, BR and TCA

For the evaluation of intra-observer and inter-observer variability, the intraclass correlation coefficient was used. The principle of the ICC calculation is a variance analysis. The resulting percentage values can be interpreted as the proportion of variance in the data that is due to differences in the observers (or observations).

3.2. LAM and BR

A random sample of 10 teeth was selected and repetitive measurements were performed by the same observer. For LAM the variation width due to the repetition of measurements was 36.0% for *P*, 13.8% for *R*, and 4.0% for *T*. For BR the variation width due to the repetition of measurements was 14.2% for *R*, and 3.1% for TM.

Additionally, a second observer made the same measurements on the 10 selected teeth. For LAM the variation width of the measurements due to two different observers was 37.2% for *P*, 13.9% for *T*, and 3.3% for *R*. For BR the variation width of

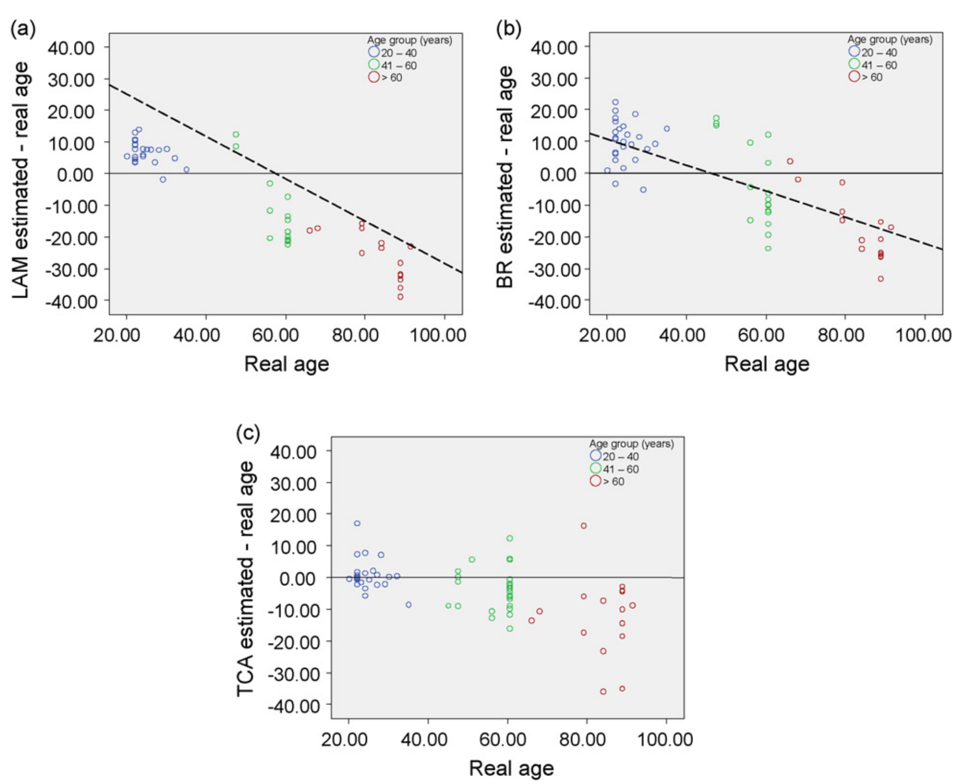

Fig. 2. Estimated age minus real age plotted against real age for LAM (a), BR (b), TCA (c). Blue data points indicate the young age group (20–40 years), green the middle (41–60 years), and red the old age group (>60 years). The continuous line through the origin represents the line on which the data points plot in case of an estimate perfectly equal to the real age. The dashed regression line in (a) and (b) refers to the residuals on the true age in the reference sample (LAM and BR, respectively).

the measurements due to two different observers was 13.7% for *R*, and 5.1% for TM of the re-tested specimens.

3.3. TCA

A random sample of 50 pictures was selected and repetitive assessments by the same observer on the photographs were performed. The variation width due to the repetition of measurements was 8.1% for the line count of the re-evaluated pictures (Fig. 3a). Most of the variation was due to interindividual difference and type of tooth.

Additionally, a second observer made the same measurements on the 50 selected pictures. The variation width of the measurements due to two different observers was 4.7% for the re-tested photographs (Fig. 3b). Most of the variation was due to interindividual difference and type of tooth.

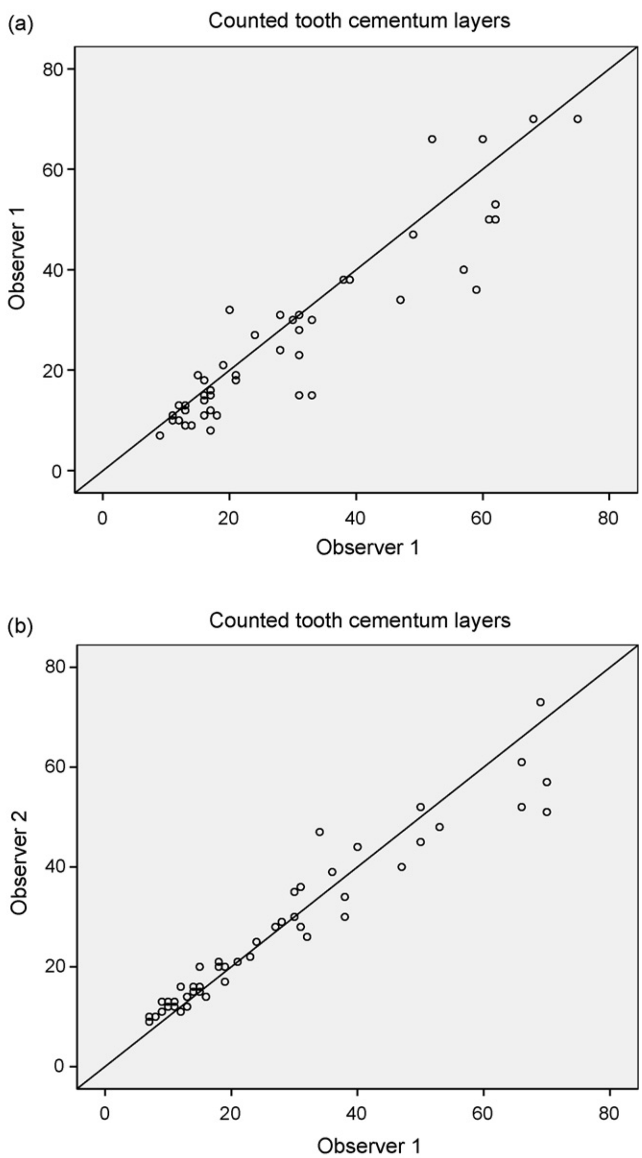

Fig. 3. Graphic representation of the intra-observer (a) and inter-observer (b) variability of TCA, based on intraclass correlation coefficients (ICC). Tooth cementum layer count of observer 1 plotted against the tooth cementum layer count of observer 1 (a) with a calculated variation width of 8.1% and tooth cementum layer count of observer 2 plotted against the tooth cementum layer count of observer 1 (b) with a calculated variation width of 4.7%.

3.4. Comparison of LAM, BR and TCA regarding performance in age at death estimation

Fig. 2 shows a graphic presentation of the data where the real age is plotted on the *x*-axis and the estimated age (or its difference to the real age) is plotted on the *y*-axis. The black line through the origin represents the line on which the data points would plot if the estimation of age yielded an estimate perfectly equal to the real age. The dashed regression line refers to the residuals on the true age in the reference sample (LAM or BR). This line serves as a tool to discover differences in the regression coefficients of the original sample (of LAM and BR, respectively) and the present sample.

The plots show that by means of LAM an overestimation of age in the young age group (20–40 years) was observed while the majority of specimens of both other age groups, middle (41–60 years) and old (>60 years), exhibited an increasing underestimation of age (2a). It can also be seen, that specimens derived from the original sample would exhibit a more pronounced overestimation in the case of the young, but a lower underestimation in the case of the middle and old age groups.

BR tended to overestimate the age of specimens from the young age group while the method was more accurate in case of teeth of the middle age group, but also underestimated specimens derived from the old age group (2b). The line of the original regression seems to mostly fit the predicted age of the specimens from the present sample.

The TCA method revealed remarkably better accuracy and precision in case of young and middle age group specimens which resulted in age estimates relatively close to the real age but underestimated old age group teeth (2c).

Table 3
Mean error (ME) as bias measure for the young, middle, old age group and all ages

Bias		ME
Age group		
20–40	LAM	6.8
	BR	9.9
	TCA	0.8
41–60	LAM	−11.9
	BR	−3.7
	TCA	−3.7
>60	LAM	−26.3
	BR	−17.4
	TCA	−12.1
All ages	LAM	−8.0
	BR	−1.1
	TCA	−4.0

Positive values indicate overestimation, negative values indicate underestimation (years).

3.5. Systematic error of LAM, BR and TCA

The mean error (ME) is presented in Table 3. The ME is a bias measure that indicates the mean of all differences between the estimated age and the true, chronological age. The results showed that young age group specimens were overestimated by means of LAM with a ME of 6.8 years, whereas middle age group and old age group teeth were underestimated with a ME of −11.9 and −26.3, respectively.

The application of BR to the present sample produced a mean overestimation of 9.9 years in the young age group specimens, a ME of −3.7 years in the middle and −17.4 years in the old age groups (Table 3).

TCA yielded a mean error of 0.8 years in young age group teeth, and a ME of −3.7 and −12.1 years in middle and old age group material, respectively. These results showed that the application of TCA to the present sample found the lowest ME in young und middle aged specimens, whereas BR was able to produce age at death estimates with a ME slightly closer to zero in teeth of the middle age group.

The assessment of the ME also revealed that the methods, except for TCA, with a ME of only 0.8 years, produced an overestimation of age in the young age group, whereas all of the techniques underestimated middle and old age group specimens.

The ME in the sample analyzed as a whole (Table 3) amounted to −8.0 years underestimation for LAM, whereas the bias of the whole sample was calculated with a ME of −1.1 years for the BR method which was also less than the ME of −4.0 for TCA.

3.6. Precision of LAM, BR and TCA

Table 4 details the standard deviation (S.D.) which served as measure for the precision of the compared methods. According to the calculations LAM revealed the highest precision of the three techniques with a S.D. of 3.6 years in the age group of the young. Increasing values were found for the middle and old age groups.

BR showed its best precision in the young age group with a S.D. of 6.8. BR worked less precisely in the middle group and in the old age group teeth.

Also TCA worked most precisely in the middle age group with a S.D. of 6.9. According to the results, LAM featured the lowest S.D. in the young and old specimens of the present sample compared with the two other named techniques. The highest precision of an age at death estimate in the middle age group was achieved with the TCA method.

Analyzing all ages (Table 4), a S.D. of 9.4 years for the application of TCA was found which constitutes the lowest variability of the estimate regarding the whole sample.

Table 4
Standard deviation (S.D.) as measure for precision for the young, middle, old age group and all ages

Precision		S.D.
Age groups		
20–40	LAM	3.6
	BR	6.8
	TCA	4.8
41–60	LAM	11.7
	BR	13.3
	TCA	6.9
>60	LAM	7.6
	BR	10.5
	TCA	12.6
All ages	LAM	15.9
	BR	14.9
	TCA	9.4

3.7. The accuracy of LAM, BR and TCA

The accuracy is given by the mean absolute error (MAE) (Table 5). This value is a measure for the accuracy of the evaluated method, or how close the age at death estimates are to the true chronological age.

The calculations demonstrated that LAM worked most accurately in the young age group. The error of the age estimation increased in the middle age group specimens and even more in the old age group.

A similar trend is given for the accuracy of BR, which is given in Table 5 for the young, middle and old age groups.

By the assessment of the MAE, TCA was found to be the most accurate age at death estimation method in the young, middle, and old age groups.

Table 5 also shows the results for the accuracy evaluation calculated for all ages which revealed that LAM was the least accurate method when compared to BR and TCA.

The present data also show that the TCA method yielded the lowest error of the three evaluated methods regarding all ages.

3.8. The predictive power of age at death estimation variables

To evaluate the usefulness of each measured variable separately, several regression models were used and the R^2

Table 5
Mean absolute error (MAE) as measure for accuracy for the young, middle, old age group and all ages

Accuracy		MAE
Age groups		
20–40	LAM	7.0
	BR	10.5
	TCA	2.9
41–60	LAM	15.4
	BR	12.3
	TCA	6.4
>60	LAM	26.3
	BR	17.9
	TCA	14.2
All ages	LAM	14.8
	BR	13.0
	TCA	6.9

Table 6
Regression models comparing the usefulness of the variables of LAM (T, P), BR (TM), and TCA (TCA)

Comparison	Model formula	R^2	Significance
Model 1	True age ∼ T	0.705	
Model 2	True age ∼ T + TM	0.716	0.162
Model 1	True age ∼ TM	0.665	
Model 2	True age ∼ TM + T	0.716	0.004
Model 1	True age ∼ T + P	0.768	
Model 2	True age ∼ T + P + TCA	0.915	<0.001

Coefficients: True age ∼ T + P + TCA

	Coefficients	Standard error	t	Significance
Constant	2.284	2.519	0.907	0.369
P	0.366	0.119	3.064	0.004
T	0.265	0.068	3.885	0.000
TCA	0.708	0.076	9.325	0.000

These models are not intended for age estimation.

values were compared (Table 6). The resulting equations are not intended for age estimation purposes. First, the root transparency variable T of LAM was compared with the root transparency variable TM of BR. The comparison of the models showed no significant improvement of the R^2 after the variable root transparency (TM) as measured by BR was added to the regression model with root transparency (T) of LAM ($p = 0.162$). Thus, no additional information about the age could be gathered when applying the root transparency measurement according to BR. On the other hand, when T measured after LAM was added to a regression of age with TM, significantly more of age-dependent variation was explained ($p = 0.004$). When the age estimation according to TCA was added to the two variables of LAM, a highly significant effect could be observed ($p < 0.001$) indicating that the cementum layer count adds to the predictive power of LAM. A multiple regression analysis showed that every single variable (root height, gingival regression and cementum layer count) adds significantly to the explained variation of age, independently of the two other variables (Table 6). The multiple R^2 of 0.915 is substantially higher than any R^2 of the other models (LAM, $R^2 = 0.768$; BR, $R^2 = 0.665$; TCA, $R^2 = 0.848$).

3.9. Differences between Lamendin et al.'s original equation and the equation of the present sample

The plot of Fig. 2a shows that if age is estimated by Lamendin et al.'s method, a deviation of the point scatter from the dashed, original regression line can be observed. It was suspected that such a large deviation may be the effect of a different relationship between the true age and the age indicators (T and P) in the present sample compared to the reference sample of LAM. To clarify how much the regression coefficients differ, the same multiple regression as used by Lamendin et al. was performed on our sample of teeth. The multiple regression analysis of the original publication of Lamendin et al. [6] provided the following equation for the age at death estimation: age (years) = $0.18\ P + 0.42\ T + 25.53$. Interestingly, both our regression coefficients (T, 0.61; P, 0.69) are higher than the coefficients of LAM, but only the value of P seems to be significantly higher. The confidence intervals of our regression coefficients (T, 0.42–0.80; P, 0.32–1.06) include Lamendin's coefficient of T (0.42), but exclude the coefficient of P (0.18).

4. Discussion

The major advantage of dental age at death estimation is the fact that human dental hard tissues do not undergo remodeling processes like bone. This makes teeth less susceptible to pathological impaction and impaired growth. Moreover, due to their extreme hardness even cremated teeth were found to feature structures still useful for age at death estimation analysis [30]. A number of methods have been employed for dental age at death estimation. Unfortunately, there are only few comparisons of these methods available, especially an analysis which compares the quantification of tooth cementum annulations with commonly used macroscopic techniques is still lacking. It was the goal of the present study to evaluate the performance of three dental age at death estimation methods, LAM, BR, and TCA, but not to analyze underlying mechanisms or factors that might affect dental age estimation.

Reproducibility is a fundamental requirement for any method used in science. Therefore, the reproducibility of LAM, BR and TCA was subject to intra-observer and inter-observer variability assessment.

The results demonstrate that LAM suffers from a high variability regarding the tooth root height and the periodontosis assessment. Thus, the respective variable for the transparency of dentin is not the primary source of variability caused by measurement error.

As can be seen in Fig. 3a and b, TCA was a most reproducible method with comparatively high intraclass correlation coefficients. This leads to the assumption that the counting of cementum annulations is useful in age at death estimation, especially by reason that not the 3×3 pictures approach, but single pictures were used for variability evaluation.

In Table 3 the bias of each method is presented for each age group and for all ages. It can be seen clearly that all three methods tended to overestimate the young age group, except the TCA method which showed a very low mean error of 0.8 years. Specimens of the middle and old age groups were underestimated by all methods. Here BR and TCA showed an almost equal result in the middle, but TCA a far better performance in the old age group teeth.

The overestimation of the young age group specimens and the consistent underestimation of the middle and old age group teeth by means of LAM, which can also be seen in Fig. 2a, is partly due to the application of ordinary regression for arriving at point estimates of the true age in the original papers, and may not only be an effect of the sample. Aykroyd et al. [27] pointed out that at least part of this error is the inevitable consequence of the statistical procedure which was used to extract an estimate of age from age indicators, namely the ordinary regression with age as dependent variable. When using LAM as the age at death estimation method, all specimens were consistently estimated lower than specimens of the original population would have been. This is indicated by the regression line in Fig. 2a and suggests populational differences. Due to a multiple regression of age on transparency and periodontosis, it can be speculated that this difference was mostly caused by a lower amount of gingival regression (or periodontosis) than in the original population, but not by a differing formation rate of the root dentin transparency, as this was also confirmed by the fact that this effect cannot be observed with BR's original regression (Fig. 2b). BR used only root dentin transparency as predictor variable, but not gingival regression. The validity of this finding is certainly reduced by the fact that the present sample consists of mainly two groups, one of which is adult and male, the other of which is older and female. Any relationship found between age and age indicators may partly be a result of this heterogeneity in the sample, although the difference between the regression coefficients is quite large (see Table 6).

The dashed regression lines computed according to the formulae of Aykroyd et al. [27] may be a valid tool for visualizing the relationship between the residuals and the true age which is solely due to the methodology of achieving a point estimate of the true age by means of ordinary regression. Any interpretation of this relationship in terms of biological reasons would be completely misleading. In comparisons of age at death methods the cluster of residuals against true age may not scatter around this line and strong deviations of the point scatter from the respective regression line may indicate a different relationship between age and indicator variables in the reference sample compared to the test sample, but this has to be interpreted carefully. We advocate confirming this suspicion by actually computing the regression coefficients of the test sample and comparing them to the regression equation of the age estimation method.

Another aspect that must be considered when employing LAM or BR for age estimation is the interception of the *y*-axis. LAM overestimates younger teeth because the interception is 25.53. Therefore, teeth that were younger than 25.53 were overestimated by LAM as a matter of fact. The same applies to BR; depending on the tooth type the constant term was 9.80 to 41.50. Furthermore, in some cases teeth came from the same individual. Therefore, we cannot rule out than an eventual metabolic disturbance of an individual might has affected the results.

Previous studies on the quantification of tooth cementum annulations reported difficulties about a correct age prediction of pathologically impacted teeth but also in case of old individuals. Kagerer and Grupe [31] conducted a study on the validity of the age at death estimate carried out on pathologically impacted material and found a mean age deviation between 2.9 and 13.5 years. The amount of the mean deviation was related to the type of pathological impaction involved. Condon et al. [5], who used demineralized longitudinal cementum sections, reported a bias ranging from 3.0 to 5.8 years overestimation for the age of individuals who were younger than 40 years old. An underestimation of 3.0–7.1 years was shown for individuals who were 40–59 years old. Renz and Radlanski [32] published a very critical study about the analysis of tooth cementum annulations in 2006 although only eight premolars were included in their evaluations. However, this article recapitulates the difficulties of the quantification of tooth cementum annulations on a well-researched basis.

The results of the present study regarding LAM are in accordance with the findings of Foti at al. [33], who calculated an underestimation of 7.53 years for the male and 10.97 years for the female individuals in their sample. These authors also demonstrated an increase of underestimation with increasing age. Martrille et al. [34] also found an overestimation of young individuals, and reported an increasing underestimation with age by means of LAM. Megyesi et al. [35] tested LAM on historical skeletal material and noted a mean error ranging from 9.3 for the youngest to 46.8 for the oldest individuals. This yielded a higher error than an evaluation of the Terry collection [36], where 32.6 years was the highest mean error which was computed for the age group 90–99 years old. The authors also reported the overestimation in age of young individuals and the underestimation of the older individuals. Soomer et al. [37] included Bang and Ramms's method in their comparative study on the validity of dental ageing methods for adults. They computed a mean overestimation of 6.6 years for the whole sample and 3.5 years for the application of LAM to their specimens.

Unfortunately, previous studies on age at death estimation differ significantly concerning the sample, the implementation of the respective method, and the statistical procedures. Therefore, the published results can only be compared very roughly and in consideration of the used study design.

Precision can be defined as a measure of ''the statistical variance of an estimation procedure'' [38]. Thus, Table 4 presents the standard deviation as measure for the precision for the three methods. Here LAM was the most precise technique in young and old age group, whereas TCA was found to have a lower S.D. in the middle age class. One possible explanation for the imprecision of the TCA method might be found in measurement errors particularly in the old age group teeth. The very low standard deviation of LAM in the young age group might be due to the reason that this age class usually exhibits only little or no visible signs for gingival regression.

Following to the formulae reported by Walther and Moore [26] the mean absolute error was computed as measure of accuracy (Table 5). The results clearly indicate that TCA is the method with the highest accuracy in all age groups, and also regarding all ages as compared to LAM and BR. This finding is in line with the fact that both other methods are based on regression equations, and confirms the present procedure of TCA. Nevertheless, it cannot be ruled out that an approach with a greater number of sections will achieve results that are even more accurate. An automated image analysis could also improve the accuracy of the age at death estimate [39], although it needs to be evaluated if this is also holds true for teeth from very old individuals.

TCA is an expensive, tedious but comparatively precise technique regarding the fact that the measurement depends principally on the absolute count of lines. Unfortunately, no standardized protocol of how to prepare the sections and how to count them exists which may lead to errors in the assessment of the line count. The majority of researchers seem to recognize a pair of light and dark bands as one ‘‘line’’, although its annual deposition in humans has not as yet been proved. In practice, the quality of the line count suffers from superimposing structures, cementocytes and an optical ‘‘thinning’’ in older individuals. Concerning the preparation process it can be said that the kind of embedding medium might not be crucial, but the thickness and the cutting angle could contribute to the quality of the section. The number of sections and the line count itself do produce an effect on the result; therefore, one should carefully choose the approach. Excluding sections with poor visibility is a subjective form of sample selection, which impairs the age assessment because it depends on the individual judgement of the observer. This reduces the reproducibility and applicability of the method, as no specific rules are given for the selection of ‘‘poor’’ sections. Due to this reason it was decided to subject all sections to further analysis, which revealed that 14 sections of the whole sample could not be evaluated, mostly due to reasons such as superimposition.

The approach with three subsequent sections and three pictures per section was chosen to guarantee a standardized procedure. It could be speculated that the age at death estimate becomes more accurate, as more sections and pictures are evaluated. The effect that especially teeth of the old are underestimated by TCA could be largely due to counting errors. The cementum of specimens of the old age group appears to be not only thicker, but also much more densely packed with optically thinner lines, a phenomenon which significantly complicates the line counting.

Furthermore, the costs of a method also affect the effective implementation. The costs for a single thin-ground section for TCA amount to about 200€ and special equipment is required, a factor which might restrict the applicability of TCA. Besides the high cost nature of TCA analysis, time is also a factor that must be considered. The preparation of undecalcified thin-ground sections takes several weeks and is destructive. Therefore, despite its high accuracy TCA analysis is only limitedly applicable to museum material and is not practicable in the field situation.

LAM and BR are both macroscopic methods that are inexpensive, quick, but less accurate. The fact that the eye must decide about the dimension of the root transparency or the gingival regression makes it more susceptible to measurement error and bias. Moreover, it is inevitable that an age at death estimation by means of LAM or BR leads to an overestimation of young age group specimens and to an underestimation of middle and old age group teeth. This is mostly a result of the use of regression-based techniques of analysis for converting age indicators into estimated ages [27]. Nonetheless, both methods are non-destructive and therefore applicable to material of museum quality. Furthermore, besides a pair of sliding callipers and a constant light source no more material or processing is required. Due to this, only minimal time and equipment are needed. The handling of BR and LAM is uncomplicated and therefore allows the realization of dental age estimation under adverse conditions. An example for this is the use of the latter by the International Criminal Tribunal for the Former Yugoslavia [37], its application to victims from the war in Croatia [40].

5. Conclusion

This study aimed to increase awareness about the differing sensitivity of the dental age evaluated by death estimation methods. It is crucial to choose the suitable method for a reliable age estimation, the possibility of estimates differing widely from the real age used in research or for medico-legal purposes may be higher than is realized.

The results of the present study indicate that the assessment of tooth cementum annulations should be favoured over the methods of Lamendin et al. and Bang and Ramm, although a standardized procedure would further improve its performance and comparability. Moreover, it could be shown that a combination of tooth cementum annulations with the variables of Lamendin et al. (transparency and periodontosis, each divided by the root length) potentially leads to an increase of the predictive power beyond the capabilities of each method alone. The present type of study ought to compare the validity and applicability of three dental aging methods using a sample that was free from pathological manifestations. Therefore, we would encourage future studies that aim to assess if our results also hold true in archaeological populations that are affected by dental disease. Further research concerning the mechanisms that underlie the formation of tooth cementum annulations but also of transparent root dentin would be a great benefit for human age at death estimation.

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