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Research Article

Tooth cementum annulation for age estimation: Results from a large known-age validation study

Ursula Wittwer-Backofen [1 2 *], Jutta Gampe [1], James W. Vaupel [1]
[1]Max Planck Institute for Demographic Research, Rostock, Germany
[2]Department of Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany
email: Ursula Wittwer-Backofen (wittwer@ukl.uni-freiburg.de)
[*]Correspondence to Ursula Wittwer-Backofen, Department of Human Genetics and Anthropology, University Clinics of Freiburg, Breisacher Str. 33, D-79106 Freiburg, Germany

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Abstract

Recent research indicates that tooth-cementum annulations (TCA) may be used more reliably than other morphological or histological traits of the adult skeleton to estimate age. Until now, however, confidence intervals for age estimated by this method have not been available for paleodemographic and forensic applications. The present study addresses this problem. Based on a large known-age sample, age estimates by TCA were conducted in a blind study involving 363 teeth. Tooth-root cross sections were made using a refined preparation technique. Improved digital graphic procedures and enhancement strategies were used to produce digital images with a specially adapted software package. This resulted in high concordance between the TCA age estimates and chronological age. Assessment of the method's accuracy, as expressed by 95% confidence intervals, showed that error bounds for age estimates do not exceed 2.5 years. Sex differences, intraindividual correlations, and the effects of periodontal disease were studied. None of these indicators had a quantitative effect on the number of TCA bands when the proposed methodological standard was followed. We conclude that the TCA technique is a reliable method for estimating a subject's age from cementum annulations. Am J Phys Anthropol 2003. © 2003 Wiley-Liss, Inc.

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Article Text

The reconstruction of mortality patterns in past populations is necessary for paleodemographic analyses. The reliability of mortality reconstruction depends on individual sex and age estimates of the skeleton as a biological source of information. For decades, osteologists and paleodemographers have strived to improve methods for determining age and sex. The importance of this research was recently highlighted in a new approach to paleodemography described by Hoppa and Vaupel ([2002]).

Almost all established macroscopic methods for age estimation in the skeleton are problematic (Buikstra and Ubelaker, [1994]; Jackes, [2001]). This is because only changes in biological age can be observed in skeletons. High interindividual variability results in error margins that may reach 7 years, at best, for ages after skeletal growth is complete (Buikstra and Ubelaker, [1994]; Jackes, [2001]; Kemkes-Grottenthaler, [2002]). The problem intensifies at older ages, as individual variability of age-dependent changes in the skeleton increases. Thus, methodological problems increase with the age of the person.

It is clear, then, that an age-estimation method is needed that is less sensitive to continuous and nonquantified age-dependent changes in the skeleton.

An alternative method, based on counting the incremental lines seen in tooth-root cementum, has shown promise. We hypothesize that these incremental lines in the tooth cementum can be used as a more reliable age marker than other morphological or histological traits in the human skeleton. This hypothesis is based on the biological factors of the tooth-cementum annulations (TCA) formation known so far.

Cementum is the calcified tissue that surrounds the dentine and forms the attachment site for the periodontal fibers that link the tooth to the alveolar bone. In cementum formation, hypermineralized layers of extracellular matrix alternate with less mineralized layers. The first layer of acellular cementum is produced before the tooth erupts, and further layers are added during and after eruption. Cementum layers consist primarily of uncalcified dense bundles of collagen fibrils. These bundles later become mineralized by hydroxyapatite crystals, whose changing orientations may be responsible for the optical effect of alternating dark and translucent layers. A biological explanation for these alternating layers was given by Lieberman ([1994]) and Schröder ([2000]). They suggested that the dark lines are stop phases of mineralization during continuing growth of the fibroblasts, leading to a change in mineral crystal orientation. This pattern is visible under the microscope as a series of alternating light and dark lines or bands.

Along the axis of the tooth root there are two zones of different cementum types: the acellular cementum, that grows close to the cervical part of the root, and the cellular cementum, which mainly covers the apical part of the tooth root. In the present study, we focus on the acellular cementum, predominantly seen in the middle third of the root. It was shown that each pair of lines corresponds to 1 year of life and constitutes a biological record that can be used to estimate the age of an individual (e.g., Lieberman, [1994]; Kagerer and Grupe, [2001]). By adding the formation age of the tooth root to the number of TCA, the age at death or tooth extraction can be estimated.

Variations in cementogenesis that change the appearance of lines may be induced by different factors, including, for example, biomechanical forces, nutrition, hormonal cycle, or ecological conditions such as temperature, ultraviolet light, humidity, altitude, or pollution (Lieberman, [1994]; Halberg et al., [1983]; Kagerer and Grupe, [2001]).

The appearance of cementum lines, observed in more than 50 different mammal species all over the world, has been said to reflect the natural metabolic rhythm of seasonal changes (Laws, [1952]; Geiger, [1993]; Grue and Jensen, [1979]; Kay et al., [1984]). Seasonal rhythms in cementum annulations, as observed in the alternating dark and light bands, can be explained by the metabolism of the parat hormone, which is responsible for the regulation of the calcium blood level, interacting with vitamin D which regulates the resorption of calcium. Thus, both hormones and vitamins may interact to produce a circannual rhythm by a complex mechanism of environmental and physiochemical “synchronizers” (Halberg et al., [1983]). Many questions remain regarding the mechanisms of tooth cementum annulation and its influencing factors, particularly concerning the interpretation of seasonal increments (Stott et al., [1980]; Lieberman, [1994]; Cipriano, [2001]).

The first use of cementum in human age estimation began with measurements of width of the total cementum layer, rather than with counts of incremental lines (Gustafson, [1950]). In the early 1980s, the study of three human teeth showed that the TCA method could be applied to human teeth as it had been to other mammals (Stott et al., [1982]). Further technical improvements (Naylor et al., [1985]) led to the suggestion that TCA is superior to other tooth-based methods of age estimation in the adult skeleton (Gustafson, [1950], [1955]; Azaz et al., [1974]; Philipsen and Jablonski, [1992]).

Initially, the TCA method was applied to freshly extracted teeth, but Großkopf ([1989], [1990]) showed that the method was also applicable to historical skeletons and cremations. This was confirmed by others (e.g., Charles et al., [1986]; Condon et al., [1986]; Lipsinic et al., [1986]; Geuser et al., [1999]) and extended to forensic cases (Jankauskas et al., [2001]). These findings add further support to the idea that the number of incremental lines is a stable property, even under circumstances where other characteristics of the lines (e.g., width, degree of mineralization) have been altered by environmental or physiological perturbations (e.g., Karger and Grupe, [2001]). It was on the basis of these kinds of results that the TCA method was recently recommended as a reliable technique for age estimation in adults using skeletal materials (for an overview of TCA applications in humans, see Wittwer-Backofen and Buba, [2002]). In subadults, however, the use of microstructural analysis of enamel and dentine leads to even greater accuracy, often within days of the true chronological age (Antoine et al., [2000]).

However, some problems remain regarding the full application of the TCA method. For example, the small samples used in previous studies limited the establishment of statistical parameters needed for practical paleodemographic and forensic applications. In addition, the question of whether dental disease, particularly periodontal disease, has an impact on TCA is still open. Großkopf et al. ([1996]), for example, found no impact of periodontal disease on TCA, whereas Kagerer and Grupe ([2001]) reported that the latter pathology reduced or arrested annual cementum formation.

In the present study, we assess the TCA method in a sufficiently large, well-characterized tooth sample. Our purpose is to outline perspectives and limitations of the TCA method by calculating confidence intervals, intraindividual correlations, and the impact of periodontal disease and tooth type on the number of TCA incremental lines.

We also focus on the graphic enhancement of incremental lines as a basis for reproducible results.

DATA AND METHODS

The sample

Our sample consists of 433 freshly extracted permanent teeth collected from several dentists and dental clinics in Germany. This sample is different from the one used in a previous study (Wittwer-Backofen and Buba, [2002]): that previous sample was used as a training sample for our observers. Maxillary and mandibular incisors, canines, and premolars are included in the study. In all cases, tooth extractions were performed as part of essential clinical care. In addition to the extraction date of the tooth and reason for extraction, the records contain the patient's date of birth, sex, and ethnicity. Among 433 teeth, there are 70 teeth from 63 individuals (16.2%) that could not be counted, either because of the poor quality of the tissue following preparation or because significant irregularities in the cementum incremental lines were present. These teeth were removed from the sample in an initial phase.

Among the excluded teeth was a significantly greater number of more maxillary than mandibular teeth, which were eliminated from the sample under consideration because of irregular histology. In these cases, a wave-like course of cementum lines was observed. The cementum lines seemed to be superimposed on each other. Neighboring areas formed broad cementum bands with no regular lines. This rendered counting impossible. These teeth were removed from the sample after microscopic image scanning. Second premolars were the predominant tooth type to be excluded for this reason (Table 1). Teeth were also excluded if the contrast of cementum lines was too low. The suitability of teeth for counting was based on judgments of image quality: 16.2% of the teeth in our sample provided unsuitable images for TCA analysis, and 363 countable teeth remained in the sample and were included in the statistical procedures.

Table 1. Number of teeth in analysis

				Excluded	
Tooth by type (FDI code)	**Total analyzed (n)**	**Males (n)**	**Females (n)**	**n**	**%**
Maxilla					
11.21 (central incisor)	45	34	11	11	19.6
12.22 (lateral incisor)	54	39	15	8	12.9
13.23 (canine)	22	14	8	13	37.1
14.24 (first premolar)	22	11	11	6	21.4
15.25 (second premolar)	19	13	6	14	42.4
Subtotal	162	111	51	52	
Mandibula					
31.41 (central incisor)	56	34	22	3	5.1
32.42 (lateral incisor)	62	28	34	3	4.6
33.43 (canine)	14	6	8	0	0.0
34.44 (first premolar)	43	29	14	0	0.0
35.45 (second premolar)	26	18	8	12	31.6
Subtotal	201	115	86	18	
Total	363	226	137	70	16.2

Teeth from both men and women were included in the sample, but there are more teeth from men than from women (Table 1). Patient's ages ranged from 12-96 years, with approximately 85% of the teeth being from individuals older than 35 years. The age distribution for men and women was quite similar up to the median, but the upper 50% of the data are dispersed more widely for women (Fig. 1).

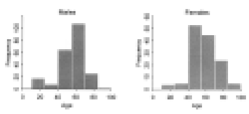


Figure 1. Age distribution in years by sex.
[Normal View 12K | Magnified View 29K]

The reasons for tooth extraction differed systematically with age, as expected. There were only slight differences in reasons for tooth extraction between the sexes.

Teeth were categorized according to their reason for extraction. The five categories were: 1) dental caries, 2) periodontal disease, 3) orthodontic care, 4) odonto-prosthetics, and 5) multiple pathologies. In practice, most teeth suffered from periodontal disease, dental caries, or both. Orthodontic therapy was a reason for extraction only for juveniles. These teeth, as well as those extracted for odonto-prosthetic reasons, can be regarded as the only healthy teeth in our sample. The presence of periodontal disease, however, did not result in exclusion from the sample. This was because we were interested in the possible impact of pathologies on TCA. This is an important issue in the analysis of historic teeth, since many such samples come from individuals with significant dental disease, especially dental caries and periodontal pathologies.

Among the 363 teeth in the sample, 229 originated from multiple extractions of 77 individuals. In most cases, 2 teeth per individual were available, although as many as 9 teeth were extracted from a single individual (Fig. 2). These multiple extractions provided the basis for establishing intraindividual correlations in TCA.

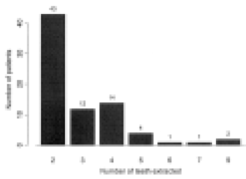


Figure 2. Frequency distribution of multiple extractions per patient.
[Normal View 14K | Magnified View 32K]

Preparation technique
Several tests indicated that the choice of optimized techniques for preparation, staining, microscope use, and counting have a significant impact on the results (Renz et al., [1997]). Different methods of preparation and of image presentation for counting may be partly responsible for the considerable variation in the reliability of TCA for age estimation that has been reported.

To estimate the intensity of periodontal regression, fresh teeth were stained in 1% Fuchsin water solution, and the maximum distance between the cemento-enamel junction and the stained soft-tissue margin was measured with a microcalliper on four surfaces of the tooth root (labial, lingual, mesial, and distal). A mean value was calculated from these measurements and used in further analysis as an “index of periodontal regression.” The greater this index value, the greater the degree of loss of periodontal attachment (i.e., more advanced periodontal disease).

After measurements were made, 70-80-μm nondecalcified transverse sections were prepared from the middle third of the root, using a Leica 1600 microtome with a diamond coated blade (for a detailed description of technical features, see Leica, [2003]). This was done after embedding the tooth crown into a block of epoxy resin, a technique designed to hold the tooth steady. Embedding the tooth root was not necessary for freshly extracted teeth, however. Next, the apical third of the root was removed, and at least three subsequent sections were cut at low speed. The unstained sections were cleaned in alcohol and mounted on slides for further analysis.

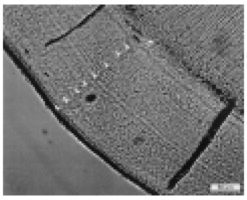
Microscopic analysis
The sections were examined in bright-field transmitted light at 200-400× magnification, using a Leica DMRXA microscope. Images were scanned using an 824 × 1,026 pixel resolution digital camera (Leica DC 250), and the image was viewed on a large-scale monitor.

The most significant technical improvements, compared to previously used methods (Wittwer-Backofen and Buba [2002]), were the use of real-time images and the digital image enhancement and counting routines. Real-time images (6-7 images/sec) allowed the complete cementum band of each forsection to be scanned, facilitating the search for an optimal focus and providing immediate quality control. At least three images per tooth were acquired and stored in a graphic database (Software package: Imagic 1000, Imagic Company Switzerland, distributed by Leica).

Images were enhanced by contrast improvement and adjusted either through the grey-scale gradation or embossing procedures. Cutting artifacts produced by the microtome were eliminated by a software macro routine via a fast Fourier transformation (Software package: Qwin, by Leica). A detailed description of the imaging system can be found in Leica ([2003]).

The resulting images showed distinct alternating dark-and-light annulation lines between the cemento-dentin junction and the periodontal ligament (Figs. 3, 4). Counting the dark lines was done manually at the monitor, based on digitally enhanced images. The dark lines were counted using the “measurement overlay.” This is a software tool that allows the researcher to mark each line by mouse-click, which is then summed, reducing the risk of human error.

Figure 3. Acellular tooth cementum of a lower canine, male, age 87.8 years. TCA age estimation of 88 years resulted from 78 incremental lines counted, added by mean eruption age of 10.0 years. Topmost arrow indicates first cementum line, followed by an arrowhead each 10 lines toward outer tooth margin. Last grouped area consists of 8



lines.
[Normal View 55K | Magnified View 162K]

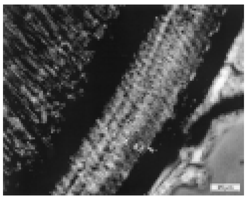


Figure 4. Acellular tooth cementum of an upper first premolar, male, age 15.0 years. Each cementum line is marked by an arrowhead, numbered by order of formation. Line number 6 is emerging, resulting in a TCA age estimation of 15.5-16.5 years, including a mean eruption age of 10.5 years.
[Normal View 35K | Magnified View 105K]

Age estimation
The counting results are based on up to four different images of one tooth. All counting was conducted by one observer (U.W.-B.). Where there was discrepancy between different counts, the mean value was used for statistical analysis. To assess the impact of tooth image quality on possible counting errors, images were classified by the observer on a three-category quality scale (1 = poor quality, with low contrast, lots of artifacts, and irregular wave patterns of cementum lines; 2 = moderate quality, with typically visible contrast, scattered artifacts, and regular lines; 3 = best quality, with almost no artifacts, good contrast, and well-defined regular lines).

To obtain an estimate of a patient's age at tooth extraction, the cementum annulation counts were added to the tooth- and sex-specific mean eruption age with its worldwide variability (Adler, [1967]; Schumacher et al., [1990]). It is not completely clear when exactly during tooth formation the cementum annulation process starts, especially in the midsection of the root. Practical considerations led to the use of mean eruption age to determine the age at formation of the first cementum ring. Tooth eruption data, based on large samples, are available for many populations worldwide and especially for Germany, where our sample stems from. Furthermore, an additive bias, which is similar for different tooth types, can be accounted for by the statistical calibration procedure.

The deviation between chronological age at extraction and TCA-estimated age is the sum of two random components: 1) individual variability in tooth eruption age, and 2) measurement errors arising from the counting procedure. These components can be assumed to act independently. Whether the measurement error can be modelled via traditional assumptions, and whether the error variance may be assumed to be constant over the whole age range, were the concerns of the present study.

RESULTS

Figure 5 summarizes the results of the counting and age-estimation procedure, separately for males and females. The top row shows a strong linear relationship between the true and the estimated ages (correlation coefficient r = 0.970 for men, and r = 0.978 for women). There are a few scattered outliers, some of which deviate markedly from true age. A deviation of more than 5 years was observed in 2.2% of the cases. Residual plots of estimated ages minus true ages are shown in the bottom row of Figure 5. Observations for which estimated age is larger than actual age result in positive differences, whereas negative values indicate age-underestimation. The two broken lines give a corridor of ±3 years as a rough assessment of the general quality of results. Neither systematic over- nor underestimation is evident over a wide range of ages. The only exception is for ages above 70, where a slight tendency of underestimation seems to be present for women. The extreme outliers are scattered over the age range, and do not cluster at certain ages.

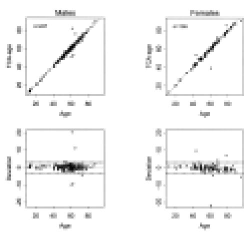


Figure 5. Top row: Estimated age vs. true age at extraction for males (left) and females (right). Thin broken line indicates identical values. Correlation is r_m = 0.970 for males and r_f = 0.978 for females (*P* = 0.075). Bottom row: Deviations between estimated and actual ages are plotted over true ages. Positive values represent overestimation; negative values stand for underestimation. Broken lines indicate an interval of ±3 years.
[Normal View 10K | Magnified View 27K]

Results for different tooth types are given in Figure 6. Minor upward bias is apparent in both men and women for the maxillary canines, and to a lesser extent for mandibular second premolars. However, no tooth position is prone to extraordinary inaccuracies.

Figure 6. Differences between true and estimated age in years by tooth position, for males (○) and females (+). Top row: Maxilla. Bottom row: Mandibula (tooth type after FDI code; see Table 1). Note that vertical axis is clipped for better visibility.
[Normal View 14K | Magnified View 37K]

Figure 7 shows the distribution of age-estimation errors as a function of image-quality index. In contrast to what might have been expected, the reliability of results is not affected by image quality. In all three index categories, the deviations center at zero and are distributed symmetrically. The most extreme outliers are seen in category 2 (intermediate image quality), and not in category 1 (images of the poorest quality).

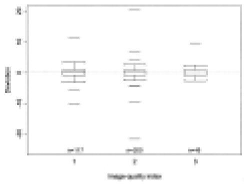


Figure 7. Deviations by categorical quality index (1 = low, 2 = moderate, 3 = good quality; for explanations, see text). Box-and-whiskers plots show central half of data as boxes with median-line inserted. Whiskers extend in both directions to last observation, which is within a distance of 1.5 times the interquartile range from first or third quartile. Values beyond whiskers are plotted as single dashes, and indicate outliers.
[Normal View 5K | Magnified View 13K]

Figure 8 shows the deviations of estimated from true ages for increasing periodontal attachment loss. The data were divided into four groups of equal size, split at the quartiles of the measured index of periodontal regression (see Preparation Technique). As the data show, and contrary to some studies (e.g., Kagerer and Grupe, [2001]), the degree of periodontal decline did not affect the accuracy of TCA. Deviations from true age were no different for teeth showing minimum periodontal decline compared to those with maximum loss.

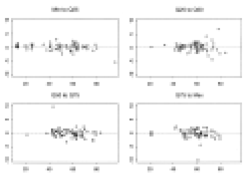


Figure 8. Deviations vs. age for four stages of periodontal decline. Cut-points for four groups are quartiles (Q25, Q50 = Median, Q75) of periodontal index. Remote outliers are clipped.
[Normal View 8K | Magnified View 21K]

Multiple teeth extracted from the same individual allowed intraindividual variability of age-estimation results to be analyzed. To investigate whether teeth from one patient showed some common bias, Figure 9 gives the deviations between true and estimated age for the 77 patients with more than one tooth available. For a majority of individuals (44 out of 77), the estimated ages cluster around the actual age. In 17 cases, age is underestimated for all teeth of a single patient, and in 16 cases the age of the individual is overestimated for all teeth. Three extreme outliers stem from individuals with multiple extractions; however, for all these individuals showing such an outlier result, the other teeth gave results within the 2s confidence band limits. There is no consistent high error for all teeth of a single individual.

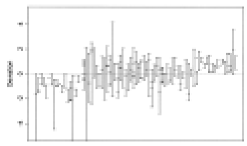


Figure 9. Deviations of TCA age from true age for those 77 individuals from whom multiple extractions were available. Individuals are ranked such that those for whom age is consistently underestimated are at left, and those for whom age is overestimated are at right of scale. Individuals with both positive and negative deviations are randomly arranged in middle. Note that vertical axis is clipped for better visibility.
[Normal View 8K | Magnified View 19K]

Calibration of age-estimation method

To estimate the unknown chronological age from TCA counts on future observations, a statistical calibration method is needed. This method has to provide both a formula for transforming a TCA count into an age estimate, and then for assessing the precision of this age estimate. If the exact number of TCA lines for an individual as well as the exact age at tooth eruption were known, then the correct age would be the sum of these two numbers. In practice, however, these two unknowns have to be replaced by appropriate estimates.

The exact number of lines will be replaced by the counted number of lines, while the unknown age at eruption will be substituted by the sex-specific and tooth type- specific mean age at eruption. Thus, deviations between the true age at extraction (or death) and the estimated TCA age stem from two different and independent sources of variation. One source of variation is the measurement error of the counting procedure; the other is the variation of the individual eruption-ages around their mean value. Multiple extractions from several individuals allow for an estimation of both variance components from the sample.

If y_{ij} denotes the estimated TCA-age based on tooth j from individual i in the sample, then $y_{ij} = C_{ij} + \mu\eta$, where C_{ij} is the counted number of TCA-lines, and $\mu\eta$ is the appropriately matched mean age-at-eruption. The exploratory analysis of the sample data above supports a linear relationship between the estimated age y_{ij} and the actual age x_i which then leads to the following model:

$$y_{ij} = \alpha + \beta\, x_i + d_i + e_{ij}.$$

Here α and β are fixed regression coefficients for which we would expect that the slope parameter $\beta \approx 1$, if the counting procedure is accurate. Both d_i and e_{ij} are zero-mean random variables, where e_{ij} is the measurement error of the counting technique, and all the e_{ij} are assumed to be independently distributed as $N(0,\sigma^2)$. The d_i represent the interindividual variability of the tooth eruption times, and are independent between different individuals and independent from the measurement errors e_{ij}. Whereas observations on two different individuals are uncorrelated, age-estimates on the same patient share a common component and are thus correlated. If $Var(d_i) = \tau^2$, then $Var(y_{ij}) = \tau^2 + \sigma^2$ and $Cov(y_{ij},y_{ik}) = \tau^2$. The intraindividual correlation is therefore $Cor(y_{ij},y_{ik}) = \tau^2/(\tau^2 + \sigma^2)$. The random-effects d_i are assumed to be normally distributed.

Modelling the individual-specific characteristics by a common random effect d_i takes into account potential correlations between observations. This is supported by observed covariation in eruption times between different teeth, which was recently documented by Parner et al. ([2001]).

Parameters in the above model were estimated by restricted maximum likelihood (REML), as provided by S-Plus (Pinheiro and Bates, [2000]). The sample was not divided by sex because preliminary analysis revealed no reason to treat males and females separately. In the presence of severe outliers, one would usually perform a regression analysis by using a robust procedure such as least-median-of-squares or an appropriate M-estimator (Rousseeuw and Leroy, [1987]). These methods take into account that outliers may have an undue influence on the estimation results and implicitly weigh down their impact. As likelihood-based approaches for variance component models are not easily amenable to robust procedures, those eight observations with the largest deviation between actual and TCA age were excluded before applying the REML procedure (McCulloch and Searle, [2001]). The results from the estimated model are given in Table 2, while the intraindividual residuals, their normal-QQ-plot, and a normal-QQ-plot for random effects are shown in Figure 10. All plots confirm the validity of the model assumptions.

Table 2. Mixed-effects model, estimating results

	Value	**Standard error**	**df**	***t*-value**	***P*-value**
Fixed effects					
$\hat{\alpha}$	0.9493	0.2530	205	3.7520	0.0002
$\hat{\beta}$	0.9839	0.0043	148	227.2584	<0.0001
	Value	**95% confidence interval**			
Random effects					
$\hat{\tau}$	0.3320	(0.0884, 1.2479)			
$\hat{\sigma}$	1.1355	(0.9824, 1.3125)			

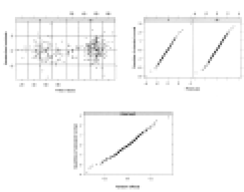


Figure 10. Top row: Intraindividual residuals and residual normal QQ-plot for linear calibration model. Results are displayed separately for males and females; however, model was estimated jointly. Bottom row: Normal QQ-plot for random effects.
[Normal View 21K | Magnified View 63K]

The estimated slope parameter $\hat{\beta}$ equals one almost exactly, which together with the small standard error of the estimate indicates that an increase in age by 1 year is translated into a 0.98-year increase in estimated TCA age. From these estimates, a total variance $\hat{\tau}^2 + \hat{\sigma}^2 = 1.3996$ and a rather small intraindividual correlation of $\hat{\tau}^2/(\hat{\tau}^2 + \hat{\sigma}^2) = 0.0788$ results.

For a new TCA-age y_0, the unknown true age $\beta \approx 1$ is estimated by inverting the regression equation, $\hat{x}_0 = \frac{y_0 - \hat{\alpha}}{\hat{\beta}}$. To determine error bounds for this estimate, an inverse prediction interval for a given level (1 - α) can be derived from the limits of the prediction interval for y_0, based on the obtained variance estimates. Formally, this is achieved by solving the following quadratic equation for x_0:

$$\frac{(y_0 - \hat{\alpha} - \hat{\beta}\, x_0)^2}{Var(y_0)} \leq t^2$$

where t is the (1 - αα2)-quantile of the tn-2-distribution. A detailed derivation of this equation can be found in Brown (1993). The interval width depends on the position of x0 relative to the age values in the calibration sample. Table 3 presents the calibration results for selected values of y0 and a level of (1 - α) · 100 = 95%. For all ages in the relevant range, the error bounds, defined by the limits of this 95% confidence interval, cover values which deviate by no more than 2.5 years from the central point estimate O_0.

Table 3. Calibration results for selected TCA ages

		95% inverse prediction inte interval	
y_0	O_0	**Lower limit**	**Upper limit**
40	39.69	37.25	42.13
60	60.02	57.54	62.50
80	80.34	77.83	82.86

DISCUSSION

In the present study, the TCA method for age estimation provided accurate results for a large sample. The sample size allowed us to conduct a detailed investigation of the impact of different individual parameters such as sex, tooth position, and presence of periodontal disease. The large sample size also allowed for a thorough statistical analysis of the results.

The results of our study demonstrate that TCA analysis may serve as a powerful method for postmortem age estimation. The accuracy is reflected in the error bounds obtained from the statistical analysis which, based on a 95% confidence level, are no more than ±2.5 years. Given the individual variability of ages at tooth eruption which are reported to have (depending on tooth type and sex) a standard deviation between 0.6-1.6 years (Adler, [1967]; after Schumacher et al., [1990]), the remaining counting error is small.

There are two explanations for the reduction of counting errors in this study. Firstly, only those images suitable for counting were submitted to TCA analysis. Secondly, and more important, the technical equipment available for the present study was significantly better than that used in previous studies. In a previous study (Wittwer-Backofen and Buba, [2002]), the observers either had to count directly while looking through the microscope (a tiring procedure), or had to use simple photographs. Three features were crucial to the improvements: 1) real-time image control that allows for selection of the most appropriate detail of the cementum band on the computer screen; 2) the digital image enhancement procedures; and 3) a software module that allows lines to be marked on the screen. In combination, these features eliminated many of the drawbacks that former studies had to cope with.

The omission of teeth of which the images were judged inappropriate for counting deserves closer consideration. The original sample of 433 teeth included all maxillary and mandibular single-root teeth. The teeth excluded were predominantly second premolars, which in fact were diagnosed as single-root teeth even when they showed a tendency towards root bifurcations. In these cases, cementum lines followed a “wave pattern” where the cementum band partly surrounds artifacts or overlays itself in undulations. They are detectable at a first quick check of the microscopic images and must be excluded from the sample, as they do not mirror regular yearly annulation lines. They look like a multiple number of interrupted line fragments, and might account for the “doubling cases” reported in previous studies. A second reason for omission was bad image quality resulting from poor quality of the tissue after preparation. If (even with the advanced technology at hand) no satisfactory contrast of the lines can be adjusted, then omitting the specimen is the only sensible thing to do. It may be argued that the high level of accuracy obtained in the present study is a consequence of excluding 16.2% of the original sample. This is true in certain respects. However, what our study shows is that *if* obviously inappropriate specimens are excluded, *then* TCA analysis produces convincing results. We want to stress once again that teeth were excluded from further processing solely because their images were not of sufficient quality and *not* because of any counting results: All images assessed to be countable were actually counted and remained in the statistical analysis.

We tested sex-specific subsamples to quantify the possible influences due to different male and female physiological conditions. Former studies showed higher error rates for teeth extracted from females. Precisely how cementum synthesis and mineralization are regulated to produce annual incremental lines is unknown, but almost certainly the cementum pattern results from the action of specific hormones and growth factors. Women experience greater fluxes in steroid hormone levels (at menarche, pregnancy, childbirth, and menopause) and calcium homeostasis (at pregnancy) than men, which could conceivably alter cementum synthesis and mineralization. However, our data do not show such an impact, as TCA errors occur equally for both sexes. The number of excluded cases corresponded exactly to the ratio of men's and women's teeth in our study. In addition, the reliability of TCA age estimations in females does not depend on age. The correlation coefficient between true age and estimated age is $r = 0.982$ ($n = 49$) for women below age 50, and $r = 0.966$ ($n = 87$) for women above age 50, which is a minor difference ($P = 0.06$).

Therefore, our results suggest that the quantitative process of cementum annulations is not influenced by menopausal disorders of the metabolic system. However, as suggested by Kagerer and Grupe ([2001]), the quality (e.g., width, apparent degree of mineralization) of the lines may be altered in women by such events as pregnancy and childbirth. This is an important issue of incremental line quality, and is the focus of ongoing studies in our laboratory.

In previous studies, the reliability of TCA age estimations in both sexes proved to be age-dependent. With higher age, inaccuracy increased for both males and females. This led to recommendations of an age-limited applicability of the TCA method for age estimation (Solheim, [1990]; Kvaal and Solheim, [1995]; Stein and Corcoran, [1994]; Lipsinic et al., [1986]). Usually this was interpreted as a metabolic disorder of higher age with the influence of periodontal regression, dental caries, or other individual characters cumulating over age.

The present study does not support these observations, since we found no age-dependent error rates. This may be due to some of the following facts. By using the scanning procedure on real-time images on the computer screen, we were able to detect the most suitable areas for further analysis, something that might have been missed by traditional ocular microscopy. Carefully applied image enhancement procedures may filter artifacts and improve the visibility of cementum lines, potentially resulting in less erroneous counting, as supported by measurement and counting software tools (Software: Imagic 1000 measurement module). This allows for counts with the aid of the computer monitor in contrast to microscopic ocular counts, which are more tedious the more lines have to be counted. As a consequence of eyestrain, reliability directly depends on the number of counted lines. Therefore, we conclude that previous inaccuracies might have been induced by less favorable technical conditions, and that problems of high-age (under) estimations can be solved by using the technical methods described in this study.

One of the more contentious discussions associated with TCA is related to the effect of periodontal disease. As the cementum annulations are interrelated with the Sharpey fibers, it is plausible that with increasing periodontal decline, the Sharpey fibers may lose their functional significance and decay owing to the reducing alveolar bone. An arrest of the cementum annulation process might occur. We paid special attention to a quantified measurement of periodontal decline. In our sample, however, we did not observe any correlation ($r = -0.075$) between the deviation of TCA age from true age and degree of periodontal decline. The outliers were distributed over the whole range of the periodontal decline. This means that even in the quarter with the highest periodontal regressions, the cementum annulations continue. From our study, we conclude that the accuracy of the TCA age estimation is independent of periodontal disease, a finding that supports the results of Großkopf et al. ([1996]) rather than those of Kagerer and Grupe ([2001]). This provides a strong argument for the application of the TCA method in archaeological skeletal samples in which most of the individuals suffered from extreme dental disease.

To analyze reliability by tooth type, we had to split our sample into rather small subgroups. However, as no sex differences in TCA estimation error were observed, different tooth types were not analyzed separately by sex. The results of our study do not suggest the selection of a specific tooth type as a favorable tooth type for age estimation by cementum annulations. All teeth in our sample from the maxilla as well as from the mandible provided results of comparable reliability. We do suggest avoiding second premolars, because they showed irregular structures of the cementum band more often than any other tooth type. If this is not considered, "doubling cases" might result. In these cases, exactly twice the number of expected lines are counted. This problem, which can affect up to 15% of a sample, was mentioned in previous studies as one of the main limitations for TCA applications (Jacobshagen, [1999]). It has been argued that the doubling of cementum lines is due to distinctive individual characteristics of the biomineralization process, which result in the apposition of two dark and light bands per year over the whole life span. In our sample, we observed no outliers that showed a doubled number of cementum bands (Fig. 5).

Deviations of TCA age estimation from true age may be caused by distinctive individual features such as an extremely early or late tooth eruption or disorders of the calcium metabolism. Both these reasons may lead to alterations affecting the whole dentition, which are detectable by comparison of results obtained from different teeth of the same individual. Our results, based on 77 individuals with multiple extractions, revealed an intraindividual accuracy which did not differ from that of independent single teeth. The individuals whose results all lie below (22%) or above (21%) the estimated age may represent early and late types of tooth eruption.

However, our sample includes 9 cases (2.5%) in which TCA age deviates by 5 years or more from true age. We carefully checked these cases for any peculiarities. Concerning the other known traits, we found no regularities in this subsample. Four cases with severe underestimation were from patients between ages 60-68 years, whereas five teeth with TCA overestimation were distributed over a broader age range. Three cases with counts too low by 10 years or more stemmed from multiple extractions. In all these cases, the corresponding results of the other one or two teeth of the same individual provided reliable results within the 2.5-year error range. This leads to the interpretation that there is no systematic factor influencing the process of cementum apposition rhythm of alternating patterns as observed under the microscope. A check of these extreme outliers with the aspartic acid racemization method of Ritz et al. ([1993]) did not provide useful results, as the teeth were treated with alkaline solutions during the maceration process, which probably caused protein degradation (Ritz-Timme, personal communication).

We cannot rule out that these cases resulted from a mix-up of teeth at the dentist's office, during preparation or during data processing. Although 2.5% is only a small percentage of the whole sample, we suggest that two teeth should be used for TCA analysis in forensic cases, and TCA should be combined with the method of root translucency of Lamendin et al. ([1992]) until the reasons for erroneous results are detected.

CONCLUSIONS AND PERSPECTIVES

Our results suggest that there is no statistically significant influence of sex, age, periodontal disease, or tooth type on the estimation quality of the TCA method, if the described preparation and analysis standard is followed. The obtained confidence band provides error estimates of less than 2.5 years. These results were obtained in a contemporary known-age sample. A subsequent study needs to show whether the same accuracy can be achieved in a historical known-age collection exposed to different environmental conditions than in a modern population.

The results of the current study extend to several aspects of historical and forensic anthropology. On the one hand, individual age estimation is improved by smaller confidence intervals, thus providing individual ages with high probability in narrow age ranges. The application of TCA age estimation improves individual age estimation and even makes age estimation possible in cases of poorly preserved skeletal fragments. For a successful application in historical populations, the influence of living conditions that severely affect the calcium metabolism in the human body still has to be tested, including severe malnutrition or specific diseases. This will be the aim of a forthcoming paper based on a historical known-age sample.

In paleodemographic applications, it is important to obtain accurate individual age estimates, especially for the oldest individuals in the population, because the highest ages achieved and the number of people reaching these ages significantly affect the calculation of different mortality parameters (Wood et al., [2002]). If the chronological age of the oldest individuals in a historical population can be accurately determined by the TCA method, this will allow us to better estimate life expectancy and the distribution of life spans within the population under study, and to compare historical populations with respect to their proportion of elderly individuals.

Finally, we recommend the application of TCA age estimation for forensic identification, since it may lead to reliable results even in poorly preserved dead bodies, and it may serve as a valuable aid for identification.

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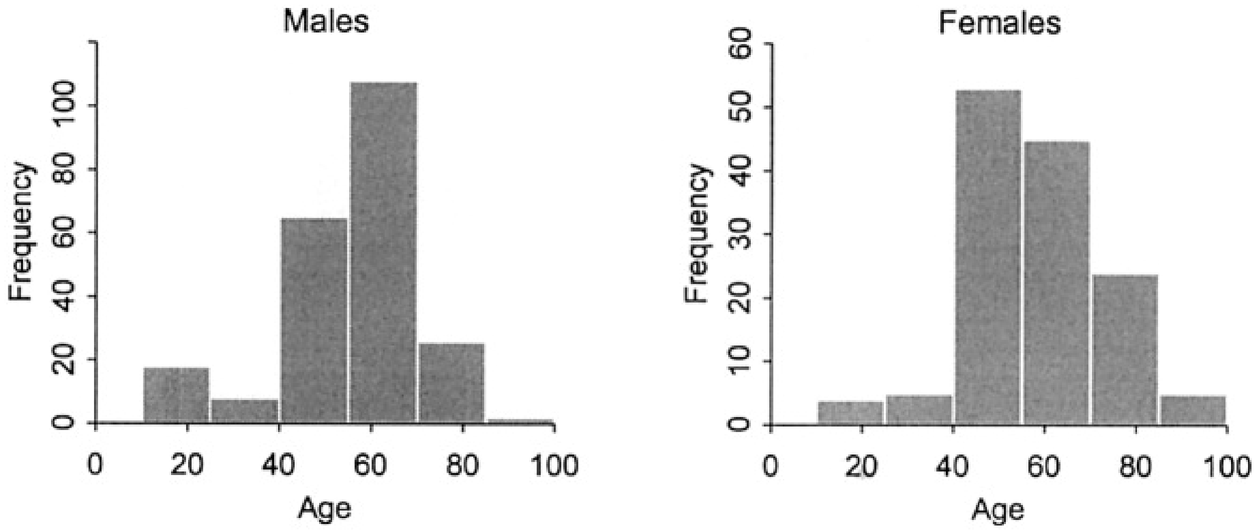

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Figure 1. Age distribution in years by sex.

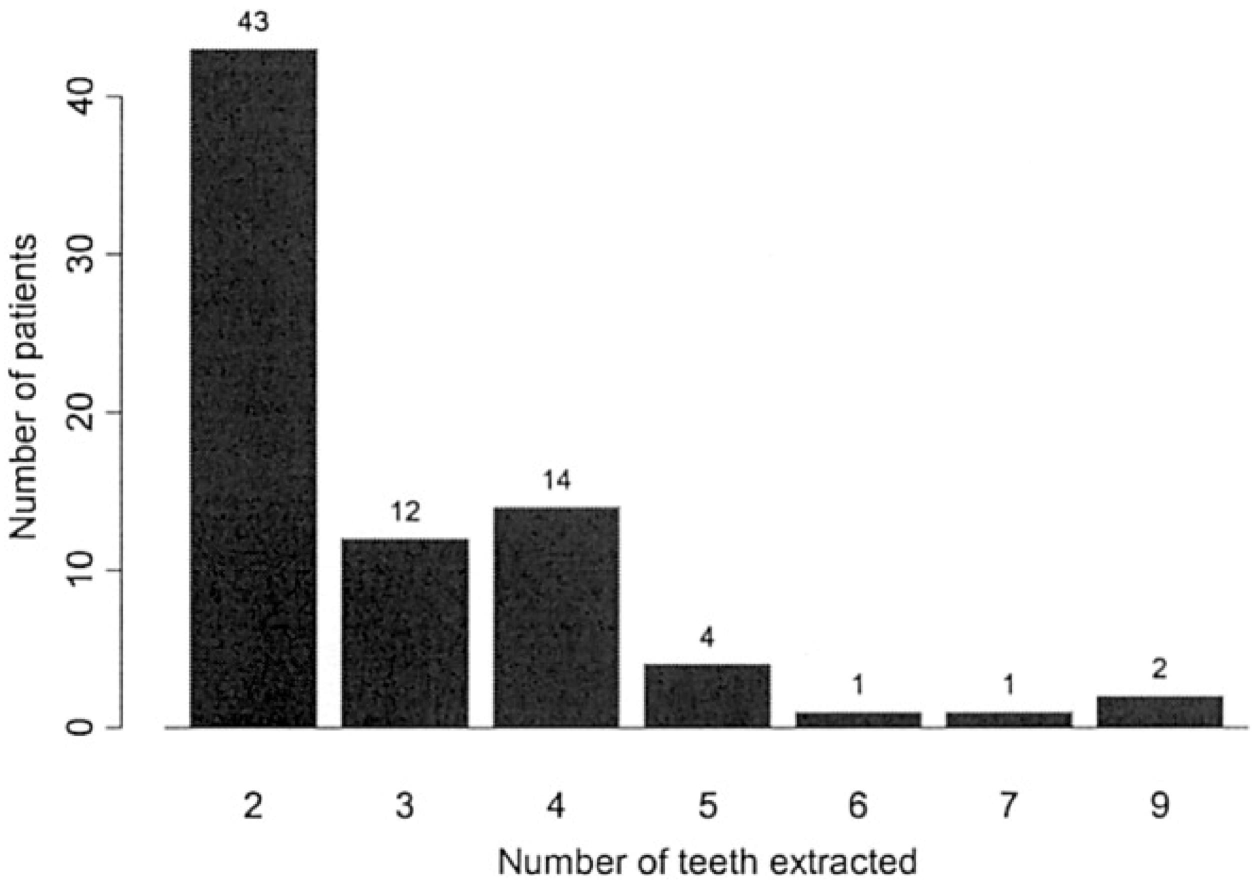

Figure 2. Frequency distribution of multiple extractions per patient.

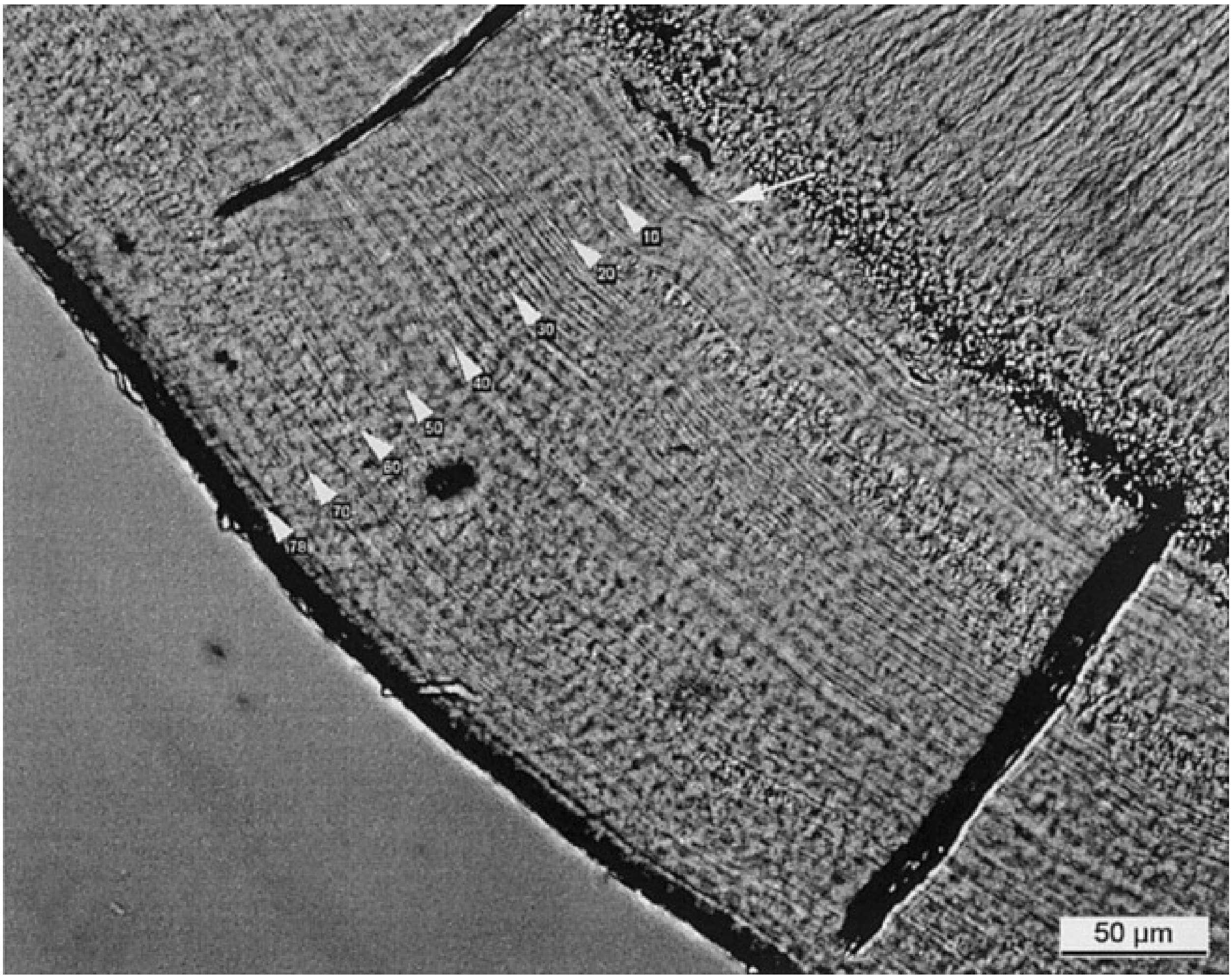

Figure 3. Acellular tooth cementum of a lower canine, male, age 87.8 years. TCA age estimation of 88 years resulted from 78 incremental lines counted, added by mean eruption age of 10.0 years. Topmost arrow indicates first cementum line, followed by an arrowhead each 10 lines toward outer tooth margin. Last grouped area consists of 8 lines.

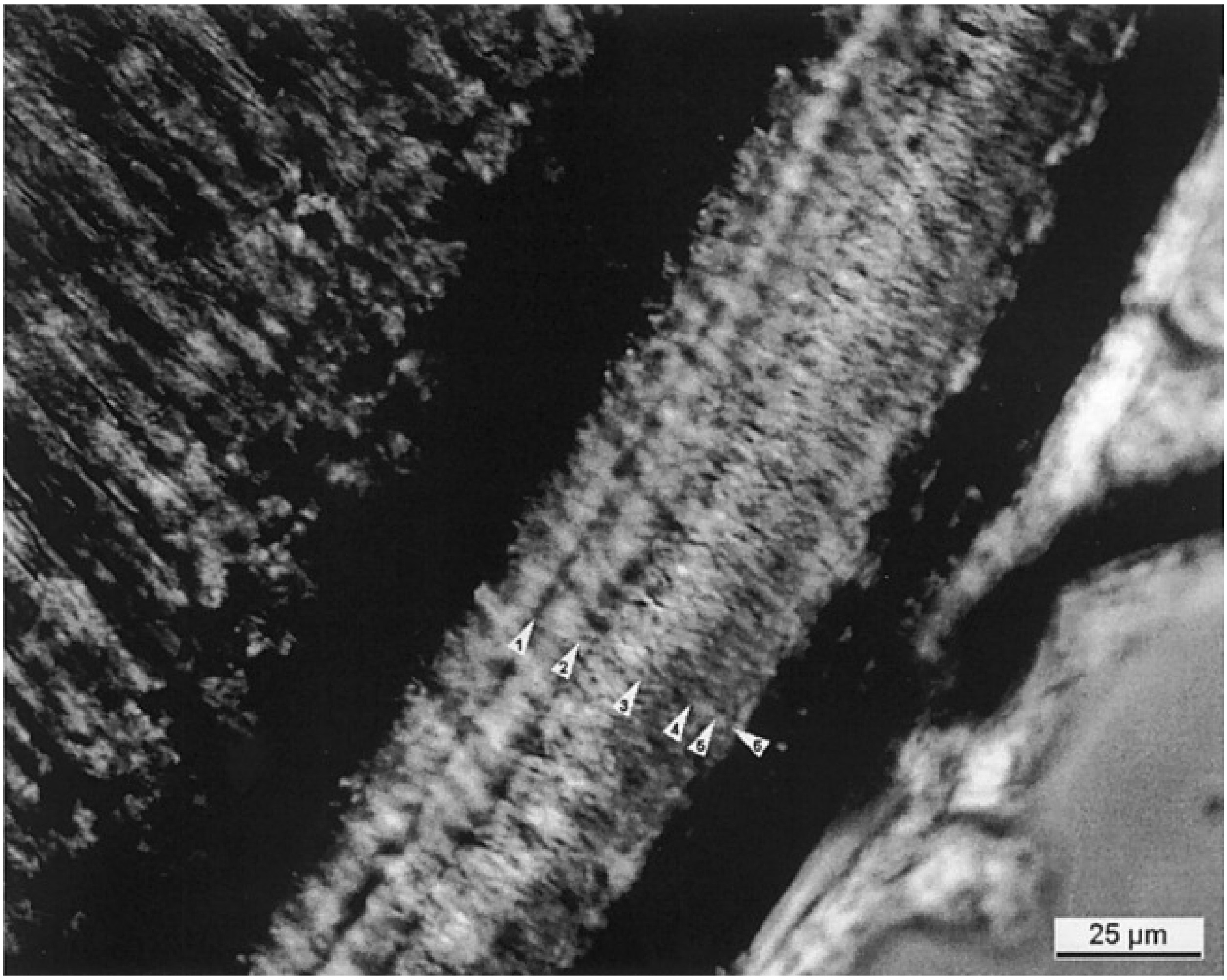

Figure 4. Acellular tooth cementum of an upper first premolar, male, age 15.0 years. Each cementum line is marked by an arrowhead, numbered by order of formation. Line number 6 is emerging, resulting in a TCA age estimation of 15.5-16.5 years, including a mean eruption age of 10.5 years.

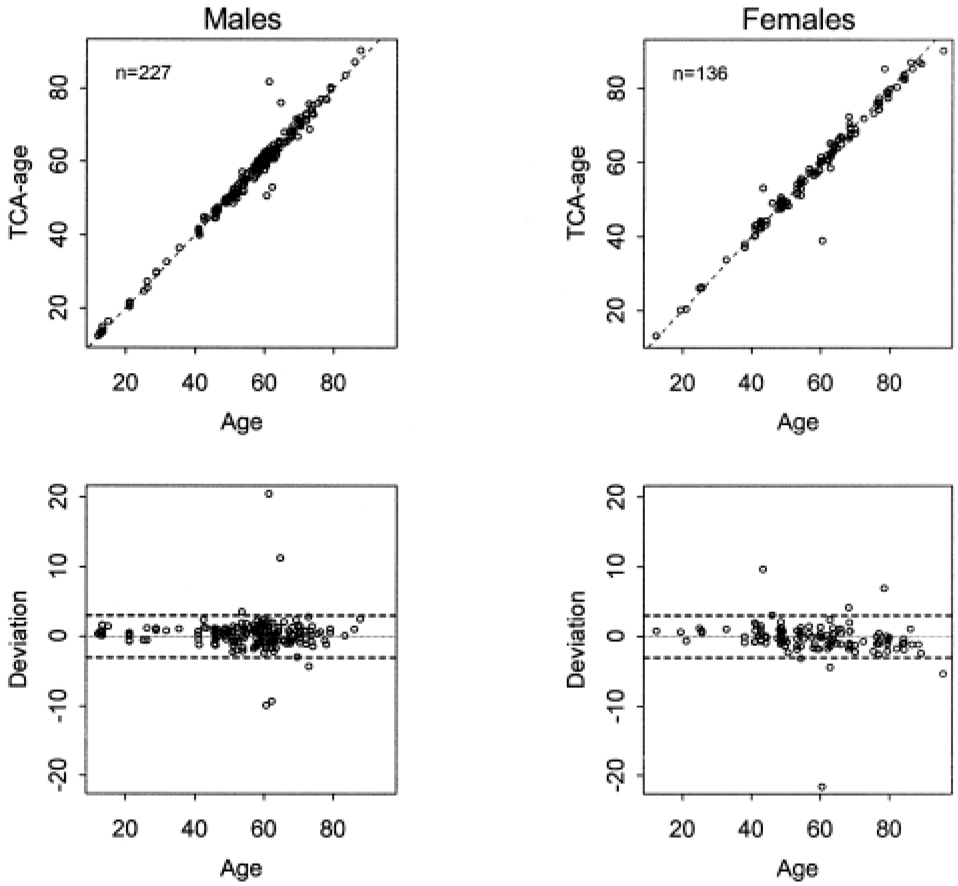

Figure 5. Top row: Estimated age vs. true age at extraction for males (left) and females (right). Thin broken line indicates identical values. Correlation is $r_m = 0.970$ for males and $r_f = 0.978$ for females ($P = 0.075$). Bottom row: Deviations between estimated and actual ages are plotted over true ages. Positive values represent overestimation; negative values stand for underestimation. Broken lines indicate an interval of ±3 years.

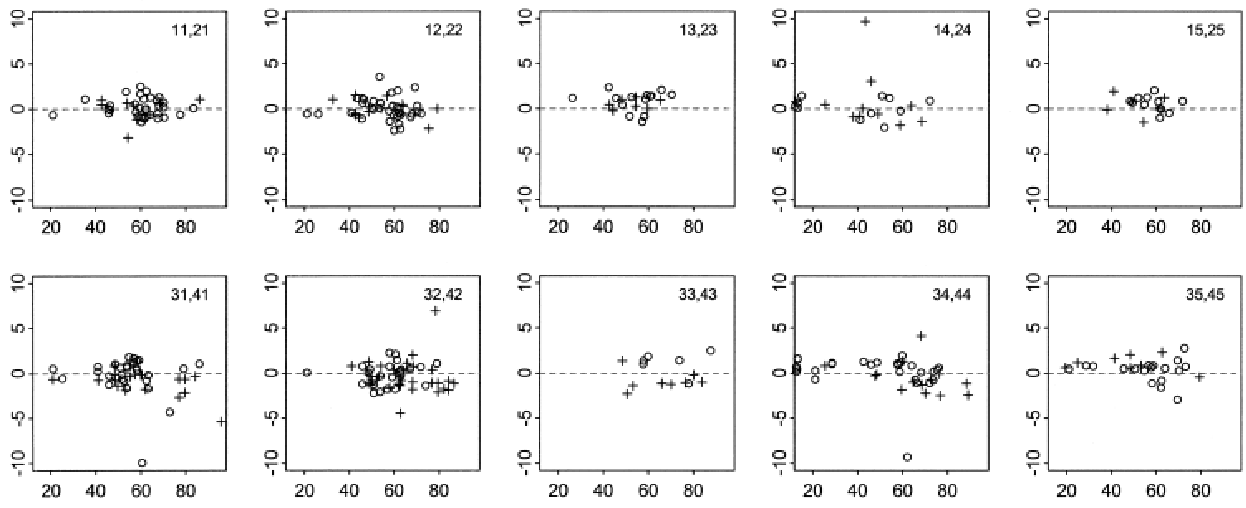

Figure 6. Differences between true and estimated age in years by tooth position, for males () and females (+). Top row: Maxilla. Bottom row: Mandibula (tooth type after FDI code; see Table 1). Note that vertical axis is clipped for better visibility.

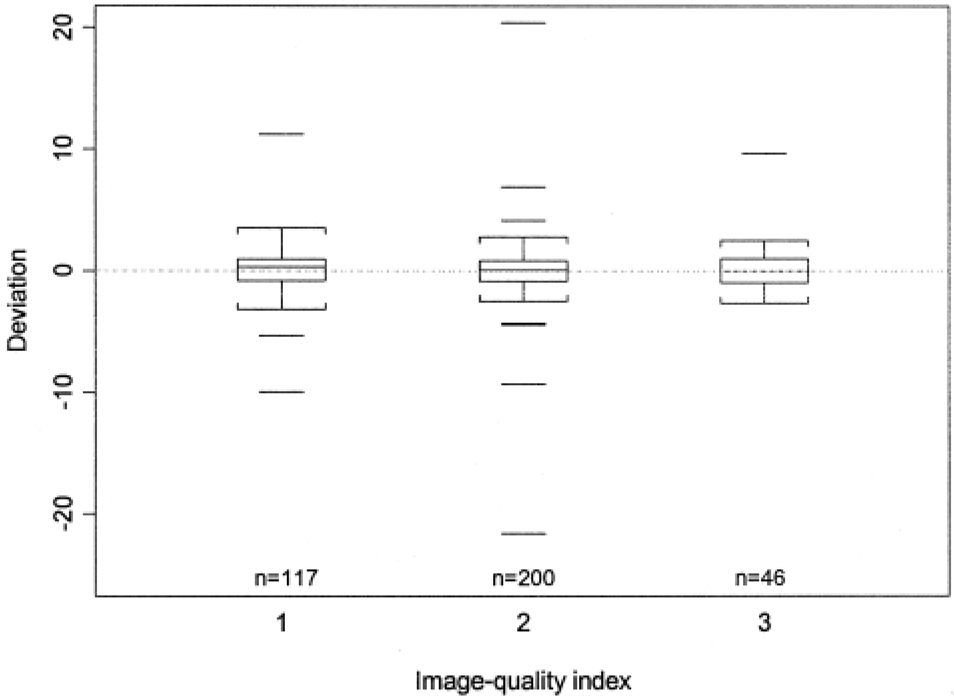

Figure 7. Deviations by categorical quality index (1 = low, 2 = moderate, 3 = good quality; for explanations, see text). Box-and-whiskers plots show central half of data as boxes with median-line inserted. Whiskers extend in both directions to last observation, which is within a distance of 1.5 times the interquartile range from first or third quartile. Values beyond whiskers are plotted as single dashes, and indicate outliers.

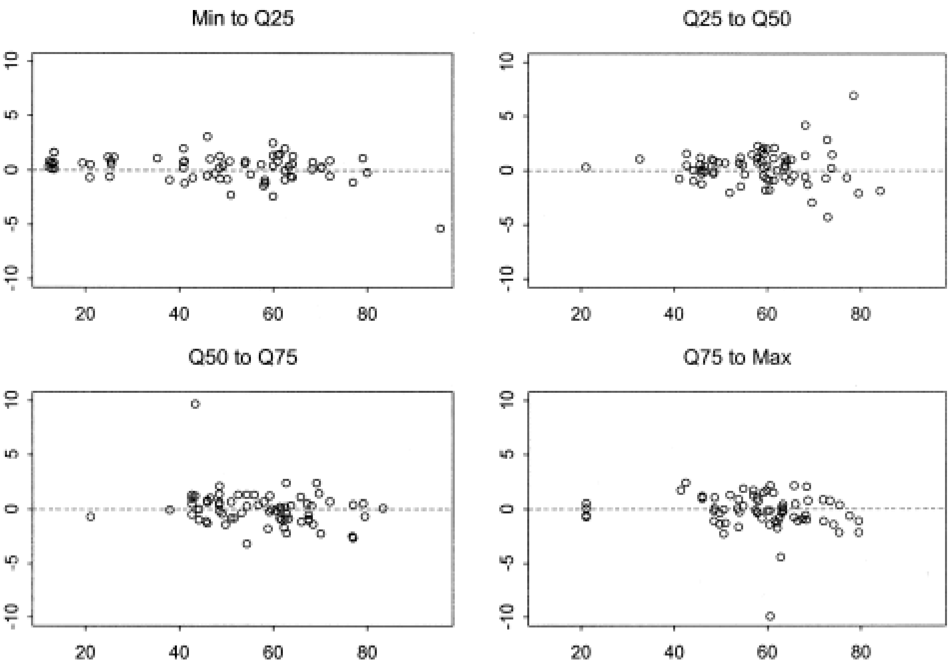

Figure 8. Deviations vs. age for four stages of periodontal decline. Cut-points for four groups are quartiles (Q25, Q50 = Median, Q75) of periodontal index. Remote outliers are clipped.

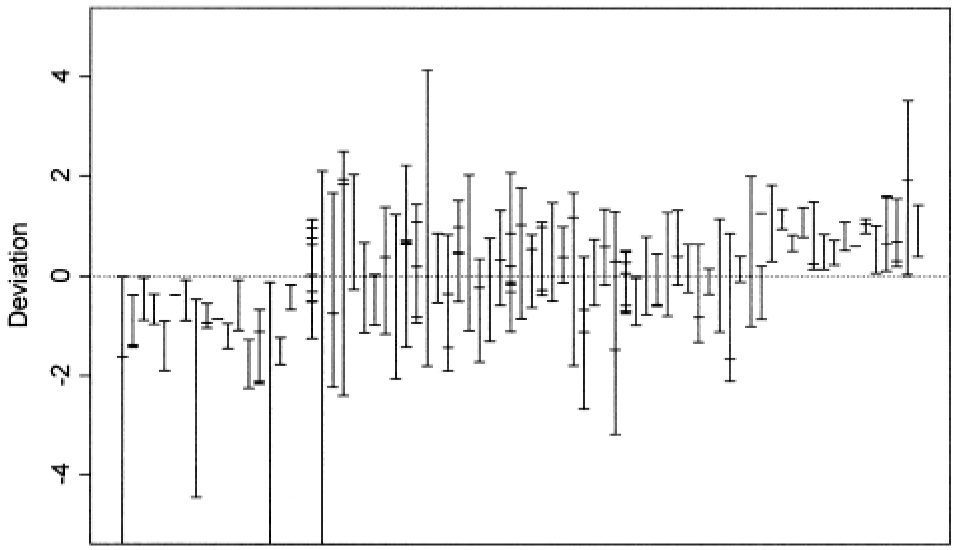

Figure 9. Deviations of TCA age from true age for those 77 individuals from whom multiple extractions were available. Individuals are ranked such that those for whom age is consistently underestimated are at left, and those for whom age is overestimated are at right of scale. Individuals with both positive and negative deviations are randomly arranged in middle. Note that vertical axis is clipped for better visibility.

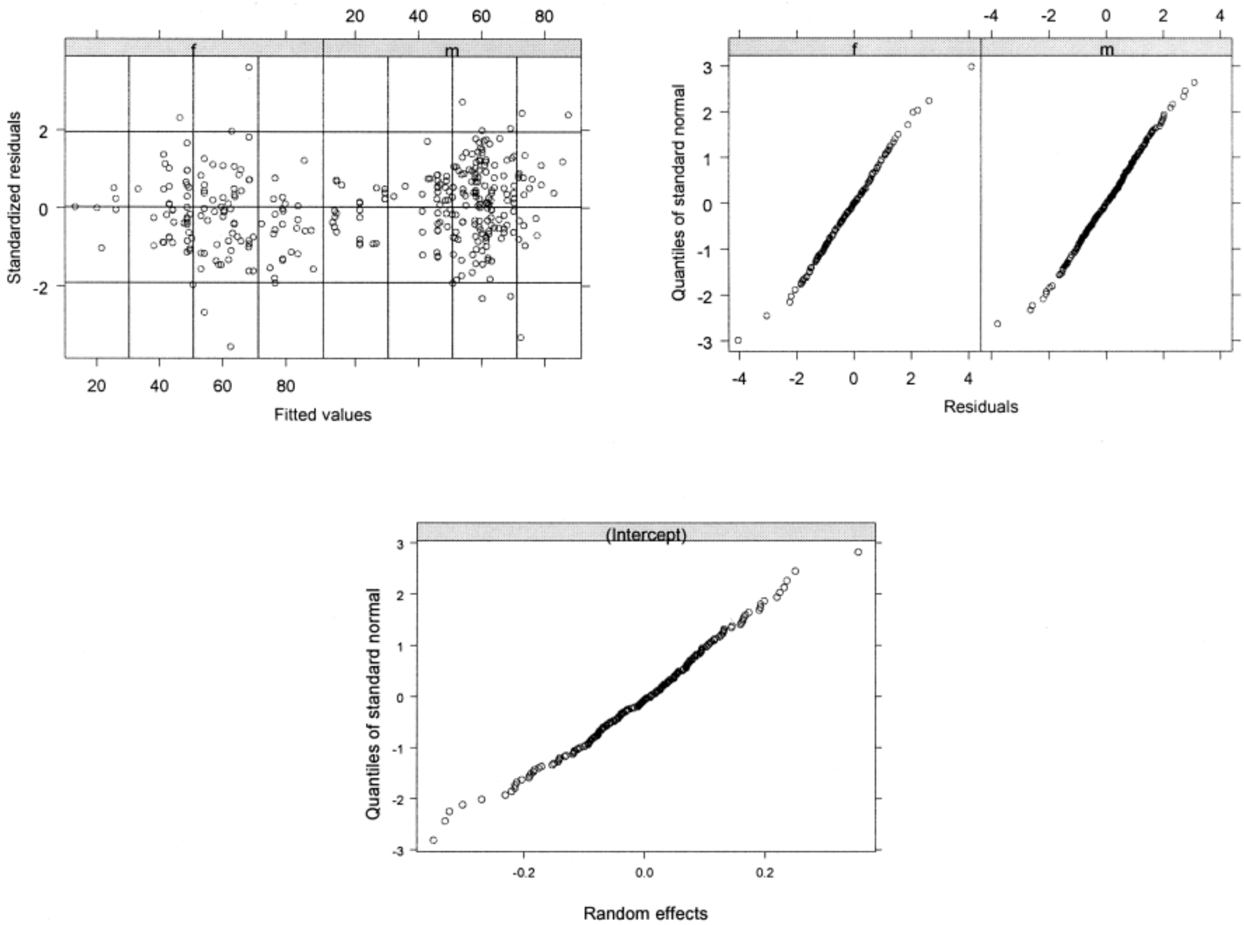

Figure 10. Top row: Intraindividual residuals and residual normal QQ-plot for linear calibration model. Results are displayed separately for males and females; however, model was estimated jointly. Bottom row: Normal QQ-plot for random effects.