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Research article

Interobserver variation affects accuracy of inference in life history studies using cementochronology

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A R T I C L E I N F O

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A B S T R A C T

Objective: Cementochronology is a method for assessing chronological age and identifying other life-history parameters (LHPs) from incremental lines of acellular extrinsic fibre cementum (AEFC) in most mammalian teeth. The aim of this study is to question the accuracy of this technique when used as a stand-alone age estimation method, and to examine how the number of observers may alter accuracy.
Design: This research is based on an extant clinical study conducted on 10 human teeth with the patients' anamnestic data. Nine observers used cementochronology to count AEFC incremental lines from 82 digital images. The counting was performed at three non-standardised areas on each image, totalling 246 counts per observer. Resultant observer counts were compared using the coefficient of variation method.
Results: The mean deviation of cementum estimated age from known chronological age of the participants in the study is 5.2 years.
Conclusion: Our study shows that further critical examination of the current cementochronology technique is essential, due to the subjectivity of line counting. The number of skilled observers in the study may improve the overall accuracy of the technique. These issues have wider implications, as many researchers rely on accurate scientific inferences being made by cementum-based studies to support or refute overarching demographic models and grand evolutionary narratives grounded by life history theory. Until this issue is resolved cementochronology should only be used alongside other age estimation methods.

1. Introduction

Teeth are an important part of the archaeological and forensic record and are widely regarded as a reliable source of information for age at death estimation and for the detection of other life history parameters. This information is important for reconstructing demographic profiles of past populations and the biological profile of an individual, and for better understanding of the pace of

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mammalian development and life history evolution more broadly [1–10]. The fact that dental mineralised tissues (enamel, dentine, and cementum) grow incrementally has encouraged researchers to deduce various life history information from teeth. Incremental layers of daily growth can be found in both enamel and dentine due to their similar developmental pattern [4], while incremental layers of annual growth can also be found in cementum, more precisely in acellular extrinsic fibre cementum (AEFC) [11]which is one of the five types of tooth cementum. This type of cementum covers the cervical two-thirds of the root and undergoes life-long deposition in a seasonal rhythmic format producing one growth layer each year (incremental line) [11]. When thin ground sections of AEFC are obtained and observed under transmitted light microscope, these increments will appear as alternating dark and light bands, each pair corresponding to one year.

Methods allowing precise identification of AEFC microstructure have profound implications for life history evolution research. For instance, crucial age at death estimations is routinely inferred from skeletal and dental data in both bioarchaeological studies and forensic science. Cementochronology, or tooth cementum annulation method as referred to in earlier studies, is a method for age-estimation and sometimes for other life history parameters reconstruction for over 70 years [12–22]. This method estimates one's age by counting AEFC incremental lines from histological sections of the individual's tooth root and adding that number to the observed tooth's eruption age. Unfortunately, this method produces results of disputable accuracy depending on the sample preparation steps, the microscopic equipment's properties, the type of tooth observed, and the counting approach employed [15,21,23–27]. As an addition to the confounding complexities of the cementum ageing technique, it has been reported that some accentuated incremental lines of AEFC correlate to specific life history parameters such as pregnancy, renal disease, skeletal trauma and fracture, parturition, and menopause [12,15,17,24,27]. These life history parameters putatively appear to change the calcium metabolism, which assumes a directly related lack of available calcium at the mineralisation front of the cementum causes formation of a visually different (i.e., accentuated) incremental AEFC line(s).

The aim of this research is to evaluate the accuracy and reliability of cementum ageing technique when used as a stand-alone age estimation and life history parameters identification method. Our assessment is qualified by our clinical study conducted on teeth of patients with known anamnestic data. Additionally, this study critically examines the often-underestimated role that the number of observers plays in cementochronology accuracy, where our null model is simply that the number of observers used in a cementum-based age estimation study does not affect the results when all observers are examining samples under the same conditions.

2. Materials and methods

The total sample size in this study comprises 10 teeth (incisors = 1; canine = 2; premolars = 4; and molars = 3), deriving form 10 participants in the study (four males and six females). Each tooth examined in this study derived from a different participant. The samples were obtained from the Faculty of Medicine, University of Priština, Serbia from patients (i.e., participants in the study) undergoing necessary dental intervention (i.e., extraction following an orthodontic treatment or/and due to caries of the crown area of the tooth) at the University Hospital Kosovska Mitrovica. All participants originated and resided on the territory of Kosovska Mitrovica municipality. Both single rooted and multirooted teeth were included of both the maxillary and mandibular dentition (Appendix 1). After the extraction, the teeth were placed in labelled vials containing physiological saline (solution of 0.90 % w/v of NaCl). All the teeth examined here were free from any visible signs of periodontal disease and radicular caries. It is worth noting that out of an initial sample size of 46 teeth, 36 teeth were excluded from this study as they did not meet the above criteria. At the time of extraction, informed consent was obtained from the participants in the study to use their teeth for the purpose of scientific research. The participants in the study filled in a questionnaire in which they disclosed information concerning on any history of renal disease, endocrinal problems, skeletal fractures and/or trauma, and pregnancies for females. The questionnaire was designed in such a manner to capture details surrounding the age of occurrence of each of the above parameters. Subsequent examination of the clinical samples obtained from each of the participants in the study provided an opportunity to critically evaluate the relationship between the cementum estimated age and the chronological age (i.e., the age at the time of tooth extraction) of the participants, including the oral evidence of other life history parameters, where available.

2.1. Sample preparation and light microscopy analysis

Prior to sectioning, the teeth were sterilized by being placed in vials, containing 10 ml of ethanol (70 %), for two days. The teeth were then air-dried and completely submerged in epoxy resin (compared to the protocol of making a resin mixture: 1 l of SpeciFix resin corresponds to ml 200 ml SpeciFix-20 Curing Agent). Up two three consecutive cross (transverse) sections, 80 μm thick, were taken from the mid-root thirds of each tooth using a saw microtome (Leitz 1600, Germany), and mounted unpolished on glass slides. Section thickness was measured with a micrometer screw-gauge (Mitutoyo Series 116, Japan). The sections were examined using light microscope (Leica DM5500). Digital photos were obtained from each section totalling 82 images (magnification 200x). All images were provided blind to the observers in the study.

2.2. Image analyses

The observers performed the incremental line counting for this study with no time limit, using the following programs to view images: Microsoft Teams Image Viewer, Preview, and Adobe Photoshop CC 2019. The AEFC incremental lines of the 82 images were counted by each observer at three non-standardised areas, totalling 246 counts each. All 246 counts per observer were recorded in a Microsoft Teams Excel Spreadsheet that all observers had access to. Each image was examined for the presence of AEFC accentuated incremental lines. These lines were identified by asking the observers to document incremental lines of varying optical effects, darker and broader than the other lines in the image. Counting of AEFC incremental lines and the accentuated lines detection were both performed as a blind study to avoid or reduce experimental biases that could have arisen from the observers' expectations. It is worth noting that the software used for the image analysis did not have a tool that keeps count of the increments, which might have contributed to observer bias. The master spreadsheet comprising the counts of all observers was compiled after each observer completed counting. This spreadsheet was then shared between the observers and supervisors to perform and check the statistical analysis in the next step.

2.3. Statistical analysis

The resultant data (Appendix 1) was then analysed to produce a standard set of descriptive statistics (Table 1), and then visualised in standard default box plots using "R" open-sourced software (version R-4.4.1) and the "ggplot2" package (version 3.5.1). This established both dispersal and central tendency of the data, and the degree of variation in and between the observations (i.e., cementum estimated age). The observer results could then be easily compared with the known chronological age, i.e., the age at the time of the tooth extraction reported by the patients themselves to their dental surgeon. This allowed us to establish the deviation in years between the observed values and the expected values for each tooth and tooth type (Table 1, Figs. 2 and 3).

Test protocols used in this study are the following.

2.3.1. variation in cementum estimated age vs. chronological age

We use Kagerer and Grupe's rigorous [17] study which set an acceptable test error threshold of 9.8 % using 80 teeth from 80 participants of 5.7 ± years variation. This protocol was developed with a large number of modern extracted teeth in arguably the least controversial and most rigorous cementum ageing study sample with known anamnestic and katamenestic data.

2.3.2. Observer Variation[1]

To better compare variation in the results between observers and their observations, we then calculated the relative dispersion around the mean for each observation made in our data, given that the average dispersion is the mean, and the absolute dispersion is the standard deviation [28]. The resultant value is known as the coefficient of variation (CoVariation), which may be expressed as a percentage.

$$CV\% = 100\sigma/\text{mean}$$

where σ = standard deviation.

Here we use the CoVariation to make a standardised comparison of the amount of variation made by and between the observers in our study. We set a standard (CoVariation) test threshold of 30 % or below to provide an acceptable amount of variation in our results [28].

1 Ethics

Informed consent was obtained from human participants in the study. Use of human sensitive data and human tissues was approved for the study by the Ethical Board of Research Executive Agency, European Commission, Brussels and the Ethics Committee of Faculty of Medicine, University of Priština at Kosovska Mitrovica (number: 09–249; date: February 24th 2015).

2 Results

The raw data collected in this study is provided in Appendix 1. This includes the following: tooth ID, sex of the participant in the study the tooth derived from, tooth type, participants' chronological age at the time of the tooth extraction, the average age of the tooth eruption, the digital image ID, and AEFC annulation counts by each observer per image added to the average eruption age of the tooth. The average age of full eruption of the tooth extracted was based on work of AlQahtani and colleagues [29].

For the entire sample series of 10 teeth, the mean deviation of the cementum estimated age from the actual chronological age was 5.2 years (Table 1). All the teeth investigated in this study were free of visible pathological conditions that could affect the precision of the cementum age estimation. Based on our results, the accuracy of cementum age estimates seems acceptable. However, cemento-chronology could not be used as a precise method for chronological age estimation.

In terms of different tooth types (Fig. 1), our most accurate results were obtained from premolars. The mean deviation of the cementum estimated age from the known chronological age for this tooth type was approximately 0.2 years (Table 1). On the other

[1] At the time this study was conducted, all nine observers in the study have had seven years university education in biomedicine including dentistry. The observers' practical expertise included years of working in a clinical environment of The Royal Dental Hospital of Melbourne. That work included daily contacts with patients, including dental procedures and treatments of dental tissues; the use of diagnostic equipment, including reading and understanding of microscopic images of different types of oral tissues. At the time this research was conducted, the observers' practical experience also included two-years of working on a cementum-focused research project (more information on this training is given in Supplementary Information 1).

Table 1
Chronological age vs. cementum estimated age per tooth type.

Category	N	Mean chronological age	Mean cementum estimated age	Mean deviation (years)
All teeth	10	44.5	39.3	−5.2
Incisors	1	68.0	45.0	−23.0
Canines	2	73.5	41.5	−32.0
Premolars	5	37.4	37.6	0.2
Molars	2	21.5	38.5	17.0

hand, inaccurate age estimates were obtained for all other tooth types. Particularly inaccurate results were obtained from canines. The mean deviation of the cementum estimated age from the known chronological age for this tooth type was approximately 32 years (Table 1). However, we need to emphasise that due to the small samples size, with a small number of teeth for each tooth type, these results are specific to this study.

A summary plot that pools all observers' cementum age estimates for each tooth in our sample is shown in Fig. 2. The dispersal of the summed observers' results is presented in a standard "R" boxplot, which is then annotated with the known chronological age of each tooth for easy comparison (chronological age = X). Each boxplot employs the standard "R" (ggplot2) default method of data dispersal, displaying the distribution of the data in quartiles, with a horizontal line delineating the median observed value, and dots representing outliers. Further summary boxplots are shown in Supplementary Information 2, in the similar format as main text but with the median, mean, and all the data plotted together. A breakdown of individual observer results by tooth is presented in a panel plot which graphically shows all the inter- and intra-observer variation (Fig. 3). In addition to the quantitative results from Tables 1 and 2, visual comparison between the individual and summary boxplots (Figs. 2 and 3) indicates that despite individual observer variation, when observer results are pooled, the central tendency of the observations (e.g., median horizontal bar) appear to move closer to the chronological age of the sample (X).

The average cementum age estimates for most teeth in our samples have generally underestimated the respective true chronological age, with sample T1 having the average cementum estimated age coincide with the chronological age, and with T30 and T34 being the only teeth where there was an overestimation in average cementum estimated age from the known chronological age (Fig. 2).

The role of interobserver error was investigated and the results are presented in Table 2. Statistical analysis included an evaluation of the amount of mean deviation of cementum estimated age from the known chronological age, the standard deviation, and the Coefficient of Variation expressed as a percentage (CoVariation) per observer. Our results showed that Observer 9 had most accurate age estimates, with the mean deviation of only one year of cementum estimated age from known chronological age. The most inaccurate estimates were obtained from the Observer 5, with the mean deviation of 20.6 years of cementum estimated age from the known chronological age. Table 2 also shows that combined average observer cementum age estimates error is very close to the average known chronological age of tooth. This suggests that accuracy of the study increases when more expert observers are involved in the study. Furthermore, six out of nine observers did not achieve our standard observational test threshold of 30 % coefficient of variation test accuracy, with all observers above 20 %. Given that we are confident that the experiment was very well controlled with a high degree of observer skill and accurate anamnestic data, this high level of observer variation when standardised in this way may indicate further problems currently exist when estimating age based on tooth cementum annual growth patterns. As tooth cementum is a relatively under-researched tissue structure, we are concerned researchers may be ignoring the possibility of more complex or

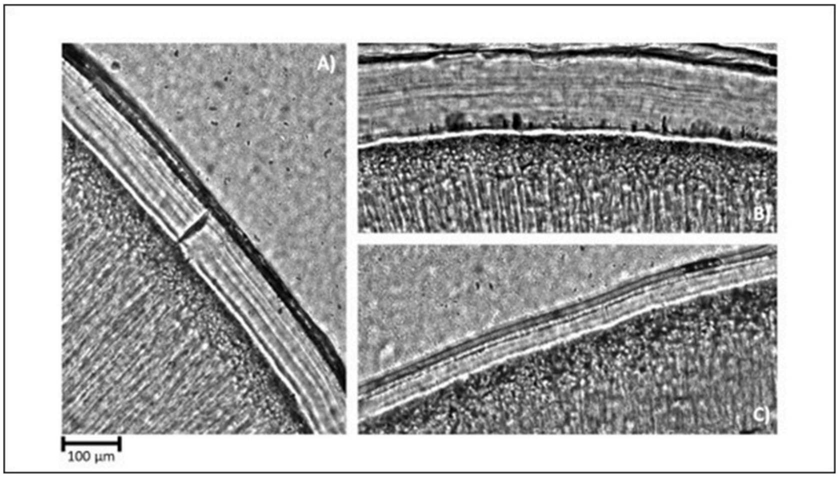

Fig. 1. Example of three AEFC micrographs from three individuals obtained form 80 µm cross-section at 200× magnification under light microscope: A) mandibular first molar (36), 30 years old female; B) maxillary first premolar (14), male, 23 years old male; C) mandibular canine (43), 66 years old female.

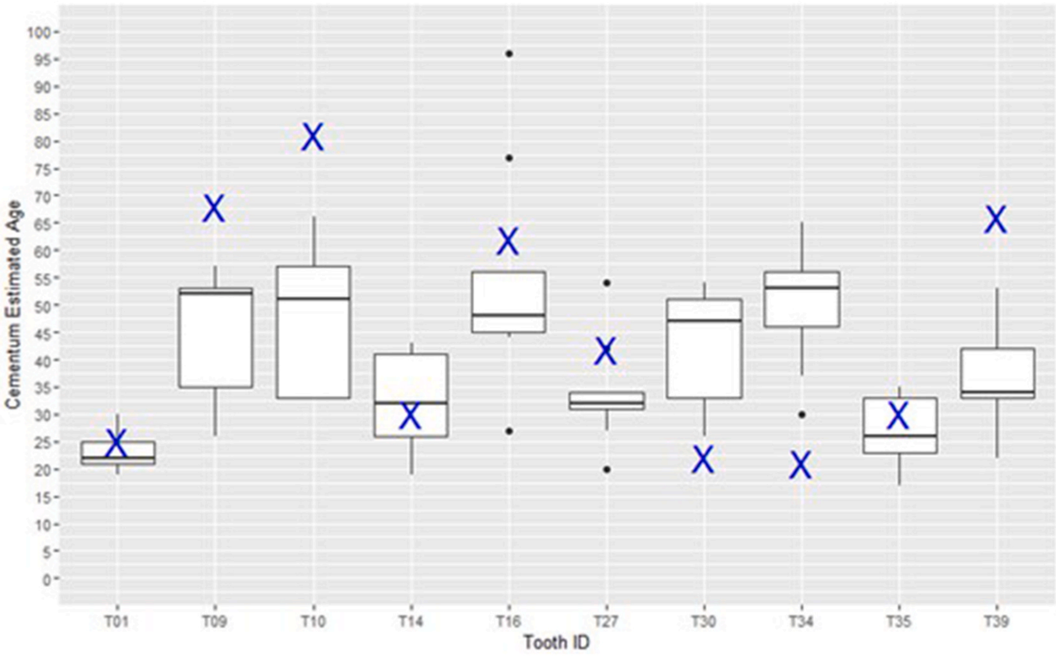

Fig. 2. Boxplots showing a standard representation of dispersal of data, pooled for all observers. Horizontal line in boxplot shows median observed Value; Dots show outliers; Blue "X" shows chronological age. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

unknown processes confounding their observed visual or chemical results, so these issues should at least be acknowledged and then better controlled for, where possible [24].

When it comes to the life history parameters identification, all observers were instructed to record the accentuated AEFC incremental lines for this purpose as explained in the section above. However, given the variation in average age estimation, including the interobserver error, no life history parameters disclosed from the participants in the study (i.e., number and timings of pregnancies, bone fractures/trauma, and metabolic disorders such as diabetes and hyperthyroidism) were successfully retrieved from the observations made by the observers in the study.

3. Discussion

Our results demonstrated the cementochronology technique is not precise in assessing chronological age using a single tooth, although it can generate a certain level of accuracy in individual age estimation. We find the average deviation of cementum estimated age from known chronological age is 5.2 years, and this level of accuracy is usually considered acceptable by other cementum studies [14,17,30]. The reasons for this deviation remain unclear. It is worth noting that the variation in the number of images among different teeth or sections in our study could affect observers' count variability. Observers in our study reported variation in regularity of AEFC incremental growth as one of the confounding issues concerning the technique's precision. Namely, the AEFC increments in our sample often differed in width and occasionally were seen to be superimposed or bifurcated - all within a single section. Some authors suggested thinner tooth sections can reduce the effects of such a phenomenon, however, this increases the risk of losing the visibility of cementum microstructure altogether [26]. In contrast to the studies that included diseased teeth [17,31,32], we excluded the teeth showing any signs of pathological conditions from our study, such as radicular caries and periodontal disease. Out of an initial sample size of 46 teeth, 36 teeth were excluded from this study. We did this to avoid the debate as to the influence of periodontal disease on the accuracy of the cementochronology. However, as stated above, the samples in this study did derive from participants undergoing necessary dental procedures. Kagerer and Grupe [17] found the highest deviation of estimated age from chronological age in teeth affected by periodontal disease, with a mean deviation of 13.5 years. On the other hand, Wittwer-Backofen and colleagues [32] observed that periodontal disease did not affect the accuracy of the cementum age estimates. They suggested periapical inflammation may affect cementum formation which can influence the number of incremental lines formed.

Other studies which used cementochronology for age estimation on samples with known age showed that the accuracy of the method varies considerably, from 2.5 to 16.1 years [17,32–34]. The study by Le Cabec and colleagues [33] inspected virtual histology of cementum using propagation phase contrast synchrotron X-ray microtomography. This technique, which has been utilised in a couple of other studies since [30,33], allows observers to visualise cementum microstructure in a such way that conventional microtomographic absorption techniques are not able to. It creates a virtual histology of the sample which allows greater freedom in choosing section thickness, the section plane and image enhancements. However, when applied to a known age archaeological human

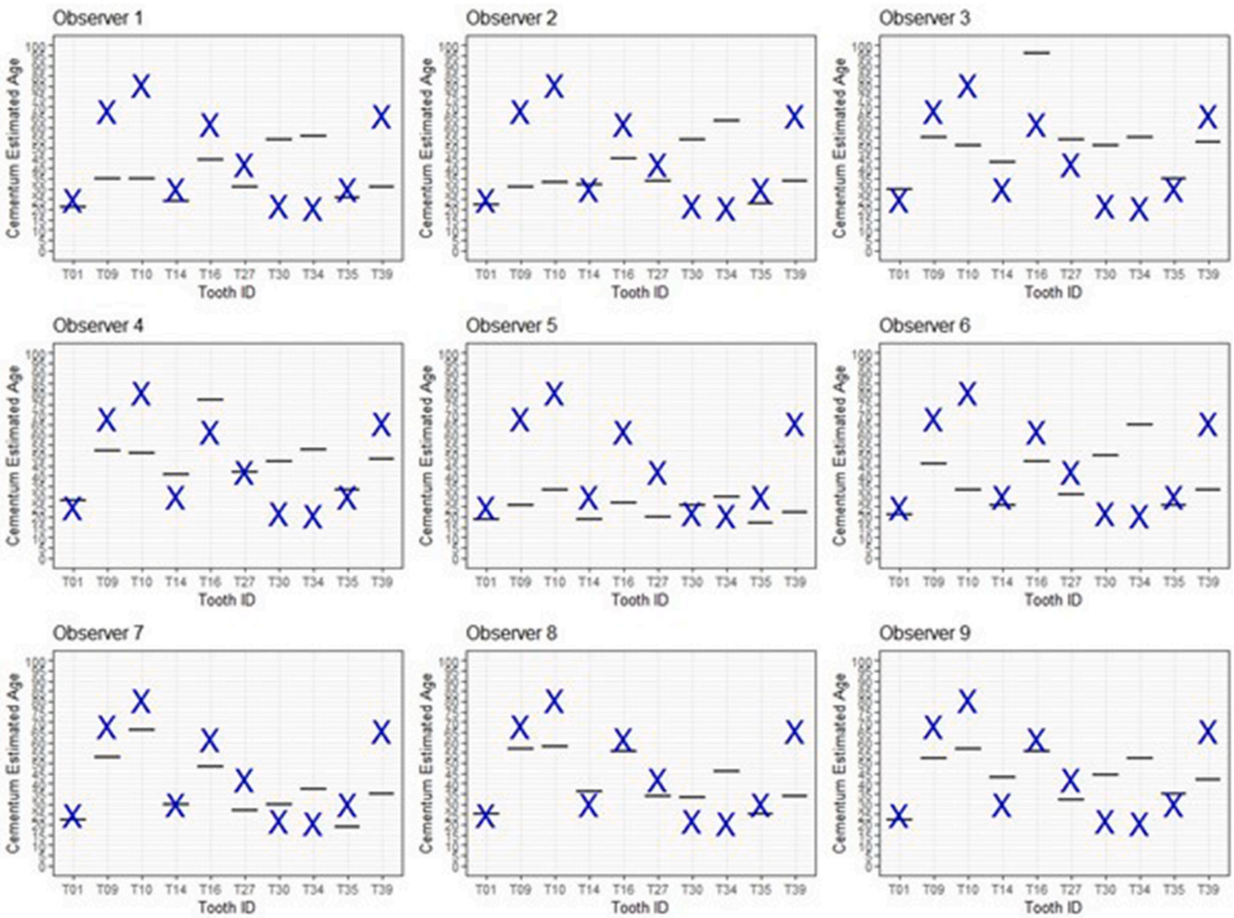

Fig. 3. Summary boxplot diagrams of dispersal of the data by each individual observer. Horizontal line in boxplot shows median observed value; Dots show outliers; Blue "X" shows chronological age. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Average cementum estimated age estimates per observer.

Category	Mean chronological age	Mean cementum estimated age	Mean deviation (years)	Standard deviation	CoVariation (%)
All observers	44.5	39.4	5.1	13.1	33.1
Observer 1	44.5	35.7	8.8	12.4	34.8
Observer 2	44.5	37.1	7.4	16.8	45.2
Observer 3	44.5	52.3	−7.8	12.6	24.2
Observer 4	44.5	47.2	−2.7	12.6	26.8
Observer 5	44.5	23.9	20.6	5.0	21.0
Observer 6	44.5	37.8	6.7	13.0	34.4
Observer 7	44.5	36.7	7.8	14.0	38.2
Observer 8	44.5	40.4	4.1	12.2	30.2
Observer 9	44.5	43.5	1.0	10.7	24.7

population to examine the degree of correlation between chronological and cementum estimated ages, this technique still generated results of an average inaccuracy of 16.1 years and an average bias towards underestimation of 15.7 years [33]. Researchers are still attempting to optimize the use of cementum-based techniques to estimate the chronological age of an individual precisely. The current state-of-the-art in the field has been recently summarised by Perrone and colleagues [27].

In terms the tooth type, our study showed that the images with the most accurate average estimated age counts derived from premolars' sections (Table 1). It has also been recorded that the regularity of AEFC incremental growth and therefore the accuracy of cementum age estimation may depend on the type of tooth observed [17,31]. Conversely, Wittwer-Backofen and colleagues [32] found that tooth type did not have a large effect on age-estimation but found minor overestimations of age in both maxillary canines and mandibular second premolars, for both men and women. On the other hand, our study has shown that canines had the highest viability in age estimation across all observers and can significantly underestimate the chronological age (Table 1). This could be due to a smaller sample size in our study and more specifically smaller number of teeth per each tooth type, as well as various ages of the participants in our study, as estimation of age in individuals above 60 are normally underestimated with cementochronology (for reasons still unknown). Our study also showed that the molars, including third molars, provided more accurate cementum-based age estimations than incisors and canines (Table 1). Given the fact that third molars have greater eruption variability than incisors and canines, one could expect that third molar cementum age estimates would be less accurate than those derived from incisors and canines. We suggest that this finding should be explored further in clinical studies downstream with a larger sample size.

Here we reject our simple null hypothesis that the number of observers does not affect the results, as the number of observers does affect the results. It appears that the more observers there are, the more their aggregated average result comes closer to the known average chronological age of all teeth (Table 1, cf. Table 2, Fig. 2, cf. Fig. 3). Although the reason for this remains unclear, our result may tentatively be related to the Law of Large Numbers, wherein the expected value gets closer as more experts participate in the experiment [35]. It is worth repeating here that all observers in this study were of the same level of experience, training, and education in histology including cementum histology. There were also no obvious external pressures imposed by the study, such as a short time limit for performing the counting of incremental lines.

In an attempt to provide increased accuracy in estimated increment counts, a standardized semi-automated increment counting procedure and additional optimization procedures for experimental settings in estimated increment counts has been implemented by Newham and colleagues [36]. To overcome barriers of interobserver variability during AEFC incremental line counting, the use of artificial intelligence (AI) has also been suggested as potentially useful where an application could be developed to perform line counting, with the aim of improving image acquisition, protocols, assessment of image quality, and interpretation [37]. On the other hand, AI may be predisposed to similar errors as humans, due to the inherent difficulty in differentiating between AEFC dark and light bands and accentuated lines [38–43]. AI cementum counting technology remains relatively unexplored. One study created a semi-automatic software registering radial thickness of cementum lines, calculating the standard deviation of greyscale values in each section from the set mean value [33]. Although results of the data subset produced improved accuracy, consolidation of parameters remains a limitation. Whilst further investigation into AI and more specifically machine learning (generative modelling) is required to improve counting accuracy, our fundamental knowledge of cementochronology needs to be improved to enable the successful incorporation of AI into our techniques.

The variation in regularity of AEFC incremental growth, reported by the observers in this study, is identified as one of the underlying reasons behind the imprecision of the age estimates. This could be due to the cementum formation patterns and less due to the visualisation technique itself. Namely, the structure and therefore the periodicity of cementum growth is largely affected by its primary function to anchor the tooth in alveolar bone via periodontal ligament [11,15,24,44–47]. The complex dynamics between the tissues of periodontium as well as the factors such as external stimuli (e.g., mechanical impact, mastication habits, type of food usually consumed, periodontal disease, etc.) and nutritional support are clearly affecting the process of AEFC annual incremental formation. Furthermore, the origin, differentiation and cell dynamics of the cementum-forming cells are still poorly understood [11]. Until the histological outcomes of these processes are fully understood it is impossible for cementum researchers to obtain precise results about histological age of this tissue.

4. Conclusions

In our study cementochronology did not yield observational results which matched the precise chronological age of the sample. This variation could result from variation in the number of images among different teeth or sections and/or a smaller number of samples per tooth type, but also from variation in regularity of AEFC incremental growth. On the other hand, our study showed that the accuracy of cementum age estimates (the average deviation of cementum estimated age from known chronological – being 5.2 years) should be considered acceptable for reconstruction of human chronological age and by extension life expectancy, a key life history trait.

Refined protocols including high resolution microscopic equipment could provide a better way of avoiding issues with cementum microstructure clarity and may contribute to a leap in cementochronology precision. For higher resolution investigative equipment to be fully utilised, calibration of equipment for this specific tissue is needed as well as a focus on developing appropriate sample preparation protocols and training for observers. If cementum and life history researchers are to go down this research avenue, more clinical studies will be needed, with larger well controlled samples. Deeper knowledge on factors influencing cementum formation is required to better interpret results based on cementochronology. On a positive note, our study has shown that cementum age estimates may be more accurate with a group of skilled observers and multiple samples, rather than a single observer using results from just a single tooth. Our results also suggest that tooth type observed could play a role in the accuracy of cementum age estimates. We found the most accurate age estimates were acquired from observation of cementum in premolars, although this could be due to smaller number of samples observed for each dental type. For future studies we suggest using larger number of observers (e.g., more than five observers per study) focused on a larger number of teeth of a single dental type, or with more specimens for each dental type per single dentition.

In our study, the identification and counting of accentuated AEFC incremental lines with the current cementochronology protocol has been shown to be unreliable in life history parameters identification such as pregnancy or disease. Identification of an accentuated line by an observer rarely correlated with the reported life history parameter for the individual/tooth observed due to variation in cementum age estimates. Lack of consensus on the definition of what constitutes an accented AEFC line does not help – it still appears to us to be quite subjective. Further investigation into the relationship between accentuated AEFC lines and life history parameters using clinical studies is clearly necessary, as known reference values are required before we can hope to understand the unknown ones present in archaeological or forensic samples. The use of generative models in cementum research might be very helpful in the future, however we suggest to the cementochronology method is neither robust nor universally standardised for secure use of AI based techniques yet. It is clearly important to control for observer variation, if we are to develop this method further.

Accurate life history parameter estimation can play a profoundly positive role in the advancement of many scientific disciplines, but researchers must remain mindful and critical of all their potential sources of error including their own observations. Our study highlights the role that the number of observers plays in cementum age estimation accuracy. As such, experimental protocols derived by small numbers of observers who are not necessarily experts in dental structures or oral anatomy are now more open to valid criticism and error than before our study. We suggest that to reduce observer error, cementum-based studies should rely on larger numbers of expert observers (>five) trained in oral anatomy and histology.

CRediT authorship contribution statement

Marija Edinborough: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Sze Long Christy Chan:** Writing – original draft, Formal analysis. **Khaled Amery:** Writing – original draft, Formal analysis. **Jasmine Ahwah:** Writing – original draft, Formal analysis. **Teema Abbas:** Writing – original draft, Formal analysis. **Aleksandra Bucki-Smith:** Writing – original draft, Formal analysis. **Vivienne Chan:** Writing – original draft, Formal analysis. **Kevan Edinborough:** Writing – review & editing, Validation, Methodology.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Marija Edinborough reports financial support was provided by European Commission. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39887.

APPENDIX 1

Table 1
Data of AEFC incremental line counts from which all calculations are derived.

Tooth ID	Sex	Tooth Type (FDI notation[2])	Age of Extraction[3] (Years)	Average Age of Eruption[4] (Years)	Digital image ID	Cementum annulation counts added to the average eruption age of the tooth								
						Observer 1	Observer 2	Observer 3	Observer 4	Observer 5	Observer 6	Observer 7	Observer 8	Observer 9
T1	Male	14 Upper first premolar	23	11.5	T1_001	19	24	32	26	19	18	22	24	23
					T1_002	22	23	32	29	19	20	22	25	23
					T1_003	21	21	32	29	18	23	19	31	23
					T1_004	21	21	26	26	17	18	21	25	21
					T1_005	21	22	29	29	21	19	22	23	21
					T1_006	21	23	31	30	20	22	23	24	27
					T1_007	21	23	30	30	19	23	28	27	21
					T1_008	21	22	33	28	18	24	23	24	22

(continued on next page)

Table 1 (*continued*)

Tooth ID	Sex	Tooth Type (FDI notation[2])	Age of Extraction[3] (Years)	Average Age of Eruption[4] (Years)	Digital image ID	Cementum annulation counts added to the average eruption age of the tooth								
						Observer 1	Observer 2	Observer 3	Observer 4	Observer 5	Observer 6	Observer 7	Observer 8	Observer 9
					T1_009	20	21	28	27	18	22	22	22	22
					T1_010	21	20	33	27	19	22	22	25	20
T9	Male	42 Lower lateral incisor	68	7.5	T9_001	36	35	62	58	32	51	60	62	60
					T9_003	25	29	37	43	20	33	41	41	39
					T9_006	38	32	63	56	26	50	62	63	64
					T9_010	38	28	56	48	24	47	49	59	44
T10	Female	33 Lower canine	81	11.5	T10_003	42	24	41	48	27	45	67	71	44
					T10_005	54	60	79	80	42	58	72	73	80
					T10_006	41	37	73	66	30	44	52	62	59
					T10_007	40	39	65	62	37	39	63	67	64
					T10_008	44	43	82	78	48	53	70	78	73
					T10_009	48	46	84	77	35	49	77	73	56
					T10_010	39	31	57	55	30	38	62	58	61
					T10_011	39	46	56	66	30	41	54	66	74
					T10_012	40	37	51	55	31	45	65	53	59
					T10_013	35	33	51	50	33	33	66	58	56
T14	Female	36 Lower first molar	30	6.5	T14_1_001	20	21	30	29	15	19	22	21	32
					T14_1_002	19	26	32	30	14	16	21	29	29
					T14_1_003	18	24	36	31	15	22	20	29	31
					T14_1_004	23	22	34	34	13	18	18	29	30
					T14_1_005	25	29	37	40	23	28	34	38	50
					T14_1_006	26	30	38	38	19	28	33	48	48
					T14_1_007	23	33	34	33	18	23	30	29	35
					T14_1_008	27	39	39	36	22	29	36	47	48
					T14_1_009	37	50	89	77	27	50	43	59	87
					T14_1_010	31	43	83	72	29	44	37	56	80
					T14_2_001	24	34	40	43	15	22	22	23	33
					T14_2_002	24	33	41	40	19	32	26	29	56
					T14_2_003	22	28	34	39	19	23	26	27	35
					T14_2_006	24	31	39	37	20	24	25	33	37
					T14_2_007	25	25	37	36	19	24	27	35	37
					T14_2_009	21	30	38	38	18	23	28	33	38
					T14_2_010	25	28	41	40	18	28	26	35	44
					T14_2_011	22	31	47	42	16	28	35	41	44
					T14_2_013	21	33	55	44	17	24	29	29	44
					T14_2_014	23	35	41	44	17	24	40	30	41
					T14_2_015	25	33	48	44	17	27	36	36	44
					T14_2_016	23	27	39	38	18	23	31	36	34
					T14_2_017	26	32	39	43	17	23	33	38	38
					T14_2_018	24	26	42	38	19	23	29	38	36
					T14_2_019	22	35	41	37	18	21	29	35	36
T16	Male	44 Lower first premolar	62	11.5	T16_002	49	60	108	87	28	50	56	63	74
					T16_003	38	41	70	57	22	40	44	45	51
					T16_004	36	39	61	43	21	37	36	39	34
					T16_006	51	56	113	91	31	54	49	66	66
					T16_007	46	49	97	93	29	50	53	61	58
					T16_009	46	58	126	95	31	53	53	58	56
T27	Female	34 Lower first premolar	42	11.5	T27_001	30	33	57	41	20	29	28	32	28
					T27_004	33	36	51	44	20	33	27	37	35
T30	Male	28 Upper third molar	22	20.5	T30_002	33	35	52	46	26	33	29	33	33
					T30_003	34	34	50	44	26	31	29	32	34
					T30_004	37	34	47	47	26	36	29	34	43
					T30_005	43	46	54	50	26	38	31	33	51
					T30_006	39	37	50	47	26	39	30	33	58
T34	Female	48 Lower third molar	21	20.5	T34_005	42	41	52	52	32	42	36	43	55
					T34_006	40	39	51	51	30	42	34	45	55
					T34_007	34	38	53	49	29	39	35	35	47
					T34_008	35	38	50	52	27	41	38	44	41
					T34_011	38	47	56	57	32	44	38	49	56
					T34_012	41	49	71	58	34	47	41	59	57
T35	Female	35 Lower second premolar	30	12.5	T35_001	26	22	34	32	17	26	18	25	35

(*continued on next page*)

Table 1 (*continued*)

Tooth ID	Sex	Tooth Type (FDI notatio n[2])	Age of Extractio n[3] (Years)	Average Age of Eruption[4] (Years)	Digital image ID	Cementum annulation counts added to the average eruption age of the tooth								
						Observer 1	Observer 2	Observer 3	Observer 4	Observer 5	Observer 6	Observer 7	Observer 8	Observer 9
T39	Female	43 Lower canine	66	11.5	T39_001	37	32	72	46	24	44	30	38	47
					T39_002	35	29	42	40	17	36	30	21	46
					T39_003	30	31	43	46	26	31	41	41	41
					T39_004	29	31	47	45	25	32	42	35	39
					T39_005	31	37	58	51	24	34	44	37	50
					T39_007	31	39	49	51	26	30	41	40	50
					T39_009	28	33	48	45	20	28	36	34	45
					T39_010	32	36	53	59	20	31	34	44	35
					T39_011	30	31	49	45	22	31	30	33	34
					T39_014	29	33	60	45	20	27	29	32	37
					T39_015	31	37	65	58	21	37	29	34	39
					T39_016	27	39	51	52	19	31	30	25	43

[2] Fédération Dentaire Internationale (FDI) notation.
[3] Age at the time of the tooth extraction = chronological age.
[4] The average age of full eruption was calculated according to AlQahtani and colleagues (2010).

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