Age-at-death-estimation in pathological individuals. A complementary approach using teeth cementum annulations

By: B. Bertrand, Gwen Robbins Schug, C. Polet, S. Nadji, and T. Colard

Bertrand, B., Robbins Schug, G., Collard, T., Naji, S., Polet, C. (2016). Age-at-death-estimation in pathological individuals. A complementary approach using teeth cementum annulations. *International Journal of Paleopathology, 15,* 120–127.
https://doi.org/10.1016/j.ijpp.2014.04.001

Abstract:

Bioarchaeologists rely on accurate estimations of age-at-death. Clearly, some pathological conditions are associated with gross morphological changes in the skeleton that could impact the effectiveness of age-at-death estimation (i.e. methods based on the pelvis, fourth rib, dental attrition, and cranial stenosis). The magnitude of this problem has not been widely studied due to a paucity of pathological skeletons of known age. We assessed age-at death for three individuals affected by bone dysplasias (achondroplasia, residual rickets, osteogenesis imperfecta) using cementum annulations and several osseous age indicators. We predicted osseous indicators that are based on gross morphological changes would yield age estimates discrepant from the cementochronology. Results demonstrated considerable differences in age estimates between morphological and histological techniques suggesting a need for additional research on the effects of pathology on the accuracy of morphological methods. Conversely, we addressed the proposition that cementum annulations will be inappropriate for age estimation in cases of chronic and severe rhino-maxillary infection and periodontitis. We assessed age-at-death for one individual with leprosy and found no indication the disease process affected cementum formation or preservation. The results of this research indicate the potential value of cementochronology in cases where skeletal pathological conditions constrain the usefulness of traditional age estimation approaches.

Keywords: Cementochronology | Age estimation | Leprosy | Achondroplasia | Residual rickets | Osteogenesis imperfecta

Article:

1. Introduction

Paleopathology is increasingly recognized as a critical tool for understanding the origin and evolution of human diseases, and it is the only means of reconstructing diversity in the etiology and epidemiological profiles of different diseases through time. Unfortunately, the field suffers from a lack of precision that limits its inferential power. At a very basic level, it is difficult to estimate age-at-death for adult skeletons, which has important impacts on demographic and epidemiological reconstructions (Séguy and Buchet, 2011) and constitutes an important part of the "osteological paradox" (Wood et al., 1992). Techniques for age estimation based on morphological changes are fundamentally limited by the typological approach to age-related changes, which assumes several morphological indicators will co-vary. An additional problem is that many of our techniques are based on Least Squares Regression, which is inappropriate for describing auto-correlated time-series data that vary in the longitudinal and cross-sectional dimension (Robbins Schug et al., 2013). Regression also suffers from a well-known centrist tendency and results in reference population mirroring (Bocquet-Appel and Masset, 1982).

These are all well-known difficulties and two important lines of research have arisen to address these issues. The Rostock Manifesto (Hoppa and Vaupel, 2002) argued for a Bayesian approach to the data. Many researchers have thus adopted probabilistic approaches to these data, including transition analysis (Boldsen et al., 2002), an increasingly popular and relatively easily employed approach that effectively alleviates some of the problems we have outlined (DeWitte, 2010, DeWitte et al., 2013). Taking a probabilistic approach to age-at-death smooths-over the lack of precision inherent to evaluating morphological changes and improves population-level comparisons (Milner and Boldsen, 2012); however, it is a statistical fix and obviously does not address the imprecision of age related changes in the morphological indicators themselves.

Another approach to this 'crisis of paleodemography' (and paleoepidemiology) is to look for more accurate and precise indicators of age in adult human tissues. Cementum annulations are the closest attribute we have to a chronometric measure of age-at-death in the human dentition. When a count of cementum annulations is added to the age of eruption of a particular tooth, this method provides the most accurate and precise estimation on which to base biocultural research (Robbins Schug et al., 2012, Wittwer-Backofen et al., 2004). The major impediment for archaeological applications is the problem of taphonomic damage (Klevezal and Shishlina, 2001, Roksandic et al., 2009), which obviously affects all age indicators. However, even if cementum annulations are applied to age only a proportion of skeletons in a prehistoric population, their use has a significant impact on the age pyramid and on demographic statistics (Robbins Schug et al., 2012). The annulations are also helpful for identifying systematic biases in other age indicators (Robbins, 2004, Robbins Schug et al., 2012).

This paper addresses this fundamental issue in age estimation. The problem we have outlined is relevant to bioarchaeological research in general (Schmitt, 2002), but it is undeniably more relevant when we are considering pathological individuals. Morphological techniques assume that the pattern and rate of maturation for individuals does not significantly vary from a reference population. It is unclear whether this postulation is appropriate for specimens exhibiting pathological or morphologically abnormal features, particularly since the standards were developed from reference samples that (logically) excluded pathological cases.

This paper provides a comparison of age estimates from cementum annulations and other morphological indicators for four individuals to address two basic questions: (1) how is skeletal age estimation affected by skeletal dysplasias? (2) Are cementum annulations useful in cases of severe periodontal disease and oral infection? We hypothesized that skeletal dysplasia will impact the accuracy and precision of osseous age indicators. This was assessed through a comparison of estimates from the pelvis, fourth rib, and cementum annulations in four individuals with achondroplasia, osteogenesis imperfecta, and residual rickets. We predicted that in cases where pathological conditions have affected bone growth or altered skeletal morphology, age estimates based on skeletal morphology will be strongly discrepant from estimates based on cementum annulations. This research does not rely on an assumption about the accuracy of estimates from different techniques, but instead evaluates the level of difference among diverse techniques for the specific dysplasias considered here.

Conversely, our second research question involves the impact of infectious diseases that affect the periodontal ligament on the formation and preservation of cementum annulations. There is a commonly held assumption that individuals affected by oral pathological conditions, including severe periodontosis, will demonstrate disruptions in cementum annulation formation and erosion of the periodontal ligament that will lessen the utility of that approach. We tested that hypothesis through an assessment of age-at-death using osseous indicators and cementochronology in an individual with severe rhino-maxillary infection and periodontitis due to infection with *Mycobacterium leprae*. We predicted that age estimated from cementum annulations would be substantially different from that estimated from osseous indicators. To assess the presence and preservation of the cementum annulations, we performed a non-destructive evaluation of a tooth from an individual with Hansen's disease (leprosy) prior to undertaking histological analysis. We predicted the cementum annulations would not be intact, a result that would suggest that destructive analysis is unwarranted and morphological age indicators would be preferred in such cases. As micro_CT demonstrated intact tissues, histology was undertaken and we evaluated the evidence as to whether severe periodontal disease disrupted cementum formation. Specific predictions for each pathological condition and its potential impact on age estimation follow.

1.1. Achondroplasia

Achondroplasia is a congenital dysplasia resulting from a mutation in the FGFR3 gene, which produces fibroblast growth receptor, a protein involved in bone formation (http://www.genome.gov/19517823). Mutations in the FGFR3 gene alter the duration of endochondral bone growth (Deng et al., 1996) and have been linked to premature cranial stenosis (Doherty et al., 2007); however, it is unclear whether the mutation affects osteoblast activity in the intramembranous bones of the cranial vault (Opperman, 2000). What is clear is that in general, achondroplastic individuals have a combination of the following musculo-skeletal manifestations: disproportionately short limbs and resulting short stature, macrocephaly, midface hypoplasia, frontal bossing, flat and deformed thorax, short rib polydactyly syndrome, premature spinal stenosis, abnormal lumbar lordosis, muscle hypotonia, and brachydactyly (Auferheide and Rodriguez-Martin, 1998:360). Thus we hypothesized that achondroplasia will affect age-at-death estimated from the 4th rib. We predicted estimates from this indicator will depart significantly from estimates based on cementum annulations and dental attrition. While endochondral bone growth is affected, the intramembranous bones develop normally and thus timing of cranial suture closure should also not be affected.

1.2. Osteogenesis imperfecta and residual rickets

Osteogenesis imperfecta (OI) refers to pathologically frail bones resulting from a deficiency in the amount or composition of Type I collagen (Ortner, 2003:492).

There are four known types of OI and 90% of cases are caused by a single, dominant mutation in COL1A1 or COL1A2, genes responsible for making the protein constituents of Type I collagen (http://www.genome.gov/25521839). As 90% of osteoid is comprised of Type I collagen, this condition affects the entire skeleton and is often associated with dentinogenesis imperfecta as well (Ortner, 2003). The condition may or may not affect endochondral formation but always manifests in limited periosteal formation, thin cortices, persistent parallel lamellar bone at the periosteal surface, and other evidence of delayed osteonal remodeling in the compact bone (Ortner, 2003). Numerous fractures will affect the morphology and articulation of the skeletal elements.

Vitamin D deficiency contributes to the etiology of rickets in childhood and of osteomalacia in adults. Vitamin D is essential for mineralization of osteoid formed during bone growth and remodeling (Pitt, 1988). In growing children, significant effects due to vitamin D deficiency occur on the forming bone structure while adults only exhibit skeletal changes relating to incomplete mineralization of bone on pre-existing surfaces (Brickley and Ives, 2008). In adults, healed childhood conditions are detectable and classic manifestations of these residual condition include: residual bending of legs, lateral narrowing of pelvis, bulging at pubic symphysis, ventral projection of sacrum, curvature of ilia, anterior angulation of sacrum, kyphosis or scoliosis and vertebral body collapse, protrusion of sternum with rib angulation and with alteration in rib neck angle (Brickley and Ives, 2008).

Both conditions (OI and rickets) affect bone growth and mineralization. Thus on a crude level, we predicted that these two conditions would be associated with changes to articular surfaces that would negatively impact the precision of age estimates. We predicted the techniques based on morphological changes to the pelvis will provide disparate age-at-death estimates when compared with cementum annulations.

1.3. Leprosy

Leprosy is caused by infection with *M. leprae*. Skeletally, the infection results in rhino-maxillary changes including erosion and remodeling of the nasal aperture; atrophy of the nasal spine; resorption of the maxillary alveoli for the incisors above the level of the anterior superior alveolar nerve; widespread periondontitis and alveolar recession; pitting and erosion of the bony palate. Postcranial changes are secondary to inflammation, degeneration, or the peripheral neuropathy that results from the disease process and often means injuries to the appendicular skeleton go untreated (Auferheide and Rodriguez-Martin, 2003:150).

Prolonged, untreated infection and inflammation in the periodontal and gingival tissue lead to destruction of the alveolar bone. In addition, circumferential hypoplasia of cementum has been recorded in leprosy patients (Ghom, 2005:655). We hypothesized that methods based on cranial stenosis and morphological changes to articular surfaces will be more consistent for estimating age-at-death in individuals with leprosy. Dental attrition was expected to be more discrepant due to AMTL (antemortem tooth loss). We predicted that cementum annulations would not be feasible for one or more of the following reasons: alveolar recession would expose the root surface to taphonomic damage; the disease process would result in the in vitro destruction of the periodontal ligament and cementum; and/or, the cementum annulations would demonstrate evidence of hypoplasia. The presence of the tissue was evaluated using microCT and when it was determined to be intact, the formation and visibility of cementum annulations was evaluated histologically. We predicted this individual might demonstrate disruptions to the annular formation of the annulations, which would limit their utility for age estimation.

2. Materials and methods

Age at death estimation indicators using gross morphological skeletal and dental techniques were applied to four anthropological cases affected by pathological conditions that affect bone growth or alter skeletal morphology. Age was estimated using standard osteological and dental methods for the end of the fourth rib (Iscan and Loth, 1986), ectocranial sutures (Beauthier et al., 2008), the pubic symphysis (Brooks and Suchey, 1990), auricular surface (Lovejoy et al., 1985), and dental attrition (Brothwell, 1981, Maat, 2000).

A skeleton exhumed at the Dunes de Coxyde abbey (Belgium, 12th–15th c.), individual H32, presents specific signs typical of achondroplasia (Polet and Orban, 2011) (Fig. 1). Sex estimation was problematic since no method has specifically looked at achondroplastic skeletons. This very well preserved individual is characterized by short stature (between 122 and 132 cm), thorax of normal height with a rhizomelic micromelia due to the shortening of bones with fastest growth and with least number of growth plates (Ortner, 2003). The skull exhibits a craniofacial form typical of brachycephalia: the occipital bone is pushed forward and the cranial vault displays a bulge on the parietal and frontal bones. Anatomic variations include thoracolumbar kyphosis, small iliac wings and narrow sacrum.

The second individual, S-515, is an adult excavated from the Saint-Amé collegiate church (A.D. 950–1797) in Douai (Northern France) (Fig. 2, a). Sex was estimated to be female based on features of the pelvis and cranium. Stature could not be estimated because of long bone distortion, but the coffin's imprints in the earth demonstrate a stature less than 120 cm. The process of differential diagnosis was challenging for this specimen. However several skeletal manifestations suggest residual vitamin D deficiency (Brickley et al., 2010, Brickley and Ives, 2008), including medial bending of the tibiae and fibulae and anterior bending of the femora; X-ray images showing a thickening of the cortical bone in the concavity (Fig. 2, b), asymmetry of vertebral bodies and rotation of the thoracic segment of the column indicating severe scoliosis (Fig. 2, c), and alteration of the rib angle and straightening of the rib shaft. The diagnosis of residual rickets is further supported by enamel hypoplastic defects and by zones of growth arrest in the femur that point to disturbances in mineral balance at young age. A diagnosis of residual rickets was preferred over a diagnosis of osteomalacia because features typical of oseomalacia were absent in this specimen, including: deformation of the pelvis and looser-Milkman zones in characteristic locations such as ribs, medial cortex of the neck of the femur and of the humerus, pubic rami, and lateral margin of the scapula (Ortner, 2003).

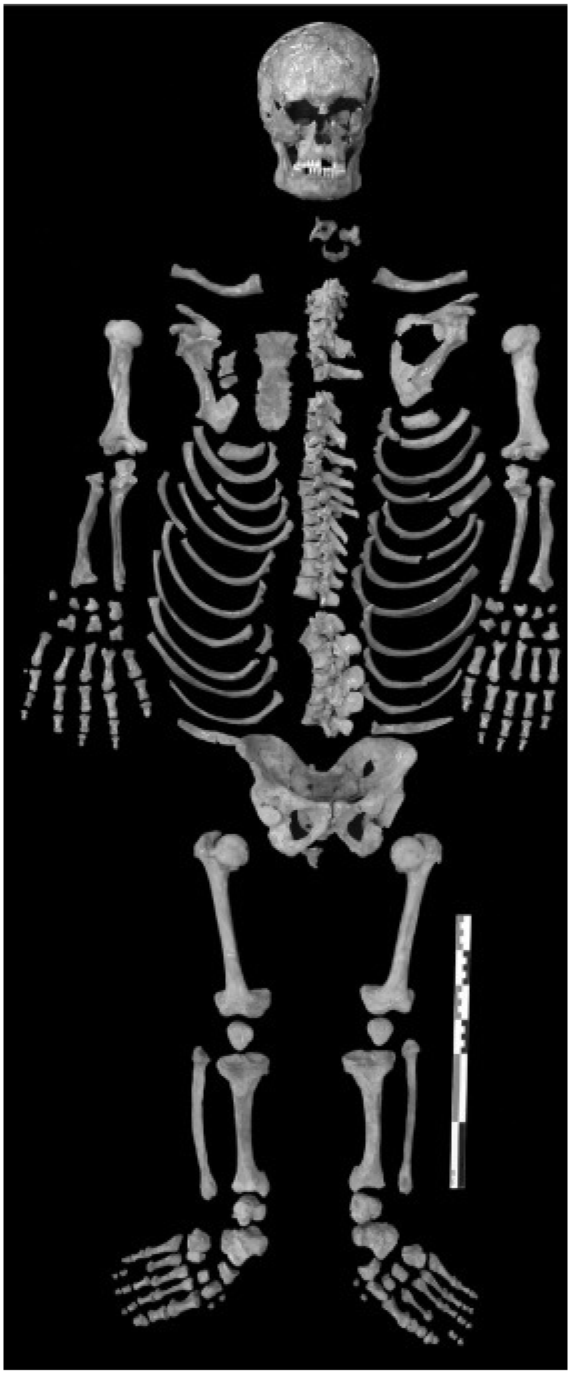

Fig. 1. Skeleton H32 exhumed at the Dunes de Coxyde abbey (Belgium, 12th–15th c.) exhibiting typical signs of achondroplasia.

Fig. 2. Specimen S-595 excavated from the Saint-Amé collegiate church (A.D. 950–1797) in Douai (Northern France) (a) showing medial bending of the tibia and fibulae with a thickening of the cortical bone in the concavity of the bends noticeable in X-ray imaging (b) and rotation of the thoracic segment of the column indicating severe scoliosis (c).

Fig. 3. X-ray imaging of the radius (a) and ulna (c) in S-593 exhumed from the Saint-Amé collegiate church (A.D. 950–1797) revealing healed fractures and severe osteopenia in comparison with individual of the same age and sex (b & d).

The third case is an adult female, identified as S-593, excavated from the 18th century Saint-Jacques cemetery (Douai, Northern France) who shows lesions indicative of probable osteogenesis imperfecta. The well-preserved skeleton shows short stature (estimated at 140 cm), noticeable bone lightness, and pathological changes include recurrent fractures. Macroscopic lesions are typical of those seen in clinically diagnosed OI patients. While the lesions expressed in this skeleton are suggestive of OI, interpretation must be made with due consideration for problems of differential diagnosis and of preservation in archaeological material. However, radiographic investigation provides support for this diagnosis as it revealed thirty healed and imperfectly healed fractures forming pseudoarthroses. X-ray imaging also showed a generalized severe osteopenia in comparison with bones from individuals of the same age and sex, preserved in the same archaeological conditions and for whom the same dental age indicator has been used (Fig. 3).

Fig. 4. BLT 1997–1 skull (Balathal – India, 2000 B.C.) exhibiting erosive lesions of the nasal aperture, including the anterior nasal spine, and resorption of the alveolar region of the maxilla associated with antemortem tooth loss.

Finally, we report the skeleton 1997–1 exhumed at Balathal, a rural outpost of the Indus Civilization (India, 2000 B.C.) who demonstrated classic signs of infection with *M. leprae* (Robbins et al., 2009) (Fig. 4). The skeleton was not complete but the elements present were relatively well preserved, including the skull and the pubic symphysis. The skeleton demonstrated remodeling of the nasal aperture, including the anterior nasal spine; exposure of the nasal neurovascular channel; alveolar resorption; evidence of inflammation on the bony palate; maxillary sinus infection; inflammatory response in the vertebrae; and periostitis on the tibial diaphysis. The character and patterning of these lesions was typical of lepromatous leprosy (Andersen and Manchester, 1992, Auferheide and Rodriguez-Martin, 1998, Moller-Christensen, 1961, Ortner, 2003, Roberts, 2002, Roberts and Manchester, 2007). Sex was estimated to be male based on features of the pelvis and cranium.

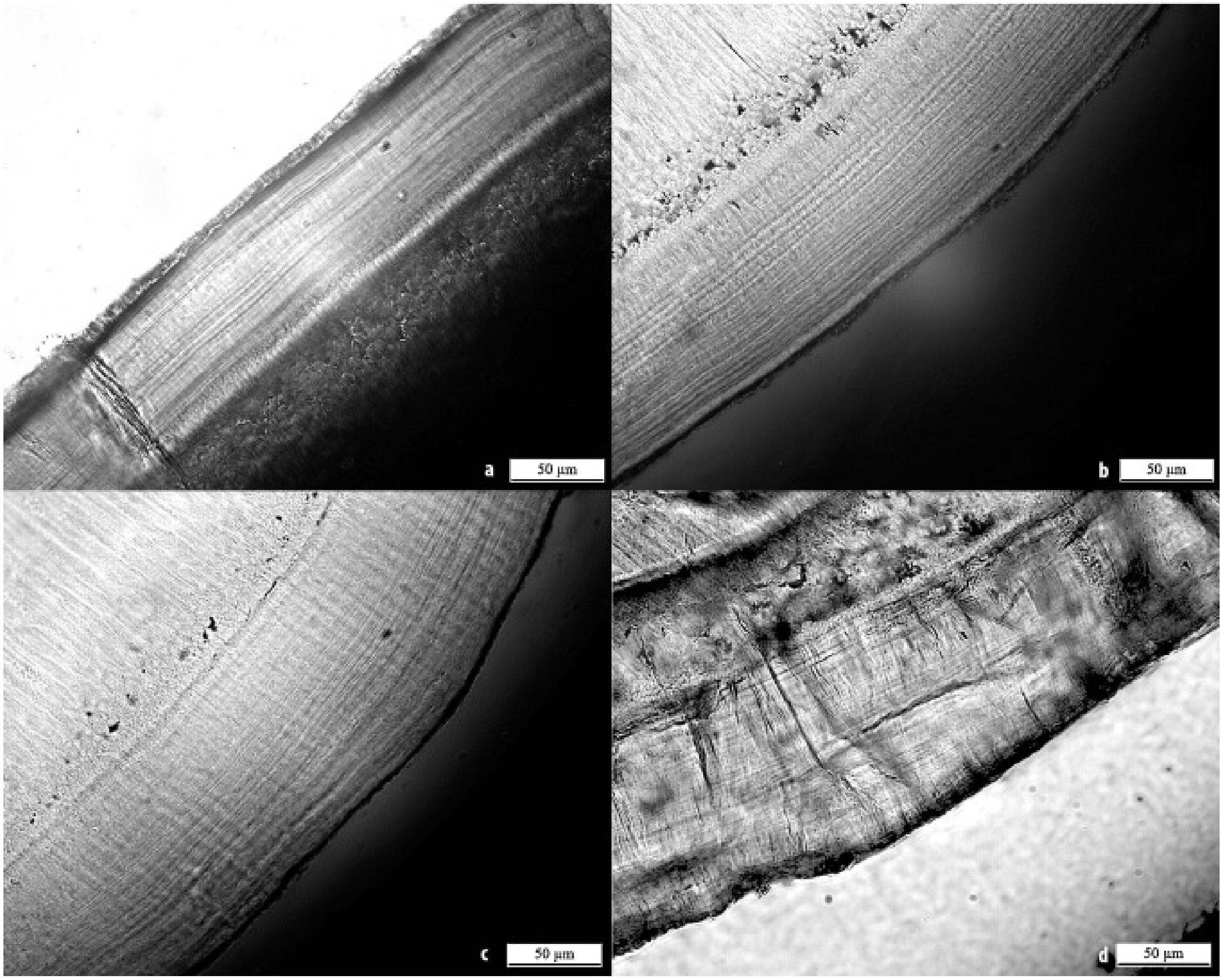

Fig. 5. Transverse sections (100 µm) of: (a) upper lateral incisor of H32 (achondroplasia); (b) upper canine of S-515 (residual rickets); (c) upper second premolar of S-593 (osteogenesis imperfecta); (d) lower right incisor of BLT 1997–1 (leprosy) showing in light microscopy the incremental lines of cementum.

For each of these individuals, cementochronology was based on the observation of the cyclic phenomenon expressed throughout lifetime of cementum appositions. Cementum is one of the four tissues that support the tooth in the jaw (periodontium), the others being alveolar bone, the periodontal ligament and gums. “Cementum annulations”, formed of hydroxyapatite crystals and collagen fibers, are generated annually on the outer layer of teeth roots. Counting the numbers of such increments was applied for the first time for age at death diagnosis in humans by Stott et al. (1982) and revealed on known-age samples a very strong correlation with chronological age-at-death (Wittwer-Backofen et al., 2004). This method, already tested on animals and humans, modern and archaeological (Roksandic et al., 2009) is very promising but still too rarely implemented.

For cementochronology, a protocol recently certified according to the ISO-9001 has been applied at the "Physical Analysis and Materials Characterization Laboratory – Direction de l'archéologie" in Douai, France. Each tooth was dried with acetone and roots were embedded in a two-component epoxy resin (Araldite® 2020) and dried in a vacuum chamber. The crowns and the upper third of the root were removed and five sequential 100 µm non-decalcified cross-sections were prepared for each tooth from the middle third of the root using a Low Speed Saw Buehler® IsoMet® fitted with a diamond coated blade. Polishing of both faces were performed in controlling the thickness to remove cutting marks and each slice was washed with distilled water. Unstained sections were mounted on slides with Canada balsam and covered with coverslip. Observation was done using a Leica DMEP microscope at 400× magnification. Segments that showed readable cementum layers were captured as JPEG images with a Leica DFC 280 fitted on the microscope. Readings were done on selected segments in Adobe Photoshop CS5. Two observers were involved in counts and the result considered was the average between the counts (Fig. 5a–d).

3. Results

Age-at-death estimates for the individual with achondroplasia (H32 – Dunes de Coxyde abbey, Belgium) yielded contradictory results. We predicted age estimates from the cranial sutures, dental attrition, and cementum annulations would demonstrate consistency and the fourth rib would be discrepant due to the disease process. Dental attrition (Maat, 2000) and the sternal 4th rib end (Iscan and Loth, 1986) gave age ranges between 25–35 years and 24–28 years, respectively. Conversely the observation of ectocranial suture stenosis (Beauthier et al., 2008) provides a minimum age of 48 years[1] and the cementum annulation counts, based on an upper lateral incisor, state that the age at death was 41.7 ± 2.5 (Table 1) (Fig. 5, a). Thus contrary to our expectations, estimates from both the fourth rib and dental attrition were strongly discrepant from the cranial sutures and cementum annulations. While actual age-at-death is unknown, it is apparent that achondroplasia did significantly impact the precision of age estimates and the results indicate caution is warranted in applying traditional methods.

Table 1. Estimates of age that obtained for the individual with achondroplasia.

Method	Skeleton part	Estimated age
End of the fourth rib (Iscan and Loth, 1986)	Rib	24–28
Dental attrition (Maat, 2000)	Teeth	25–35
Ectocranial sutures (Beauthier et al., 2008)	Cranial vault	>48
Cementum annulations (Stott et al., 1982)	Upper lateral incisor	41.7 y ± 2.5

On the skeleton with skeletal manifestations consistent with vitamin D deficiency (S-515 – Saint-Amé collegiate Church, France), we predicted pelvic indicators would be discrepant from the cementochronology due to disease impacts on pelvic morphology and locomotion. This prediction turned out to be accurate in the sense that pelvic indicators were discrepant, however there was no consistent direction for those discrepancies. The pubic symphysis (Brooks and Suchey, 1990) yielded an age of 38.2 years ± 10.9. The auricular surface of the ilium (Lovejoy et al., 1985) indicated age-at-death was 50–59 years (Table 2). The cementochronology process was based on an upper canine and a lower canine and respectively states an age at death of 49.6 years ± 3.6 and 48.8 years ± 3.6 (Fig. 5, b). Thus reliance on pelvic indicators of age for this individual significantly expanded the range of age estimates, reducing the precision.

Table 2. Estimates of age that obtained for the individual with residual rickets.

Method	**Skeleton part**	**Estimated age**
Pubic symphysis (Brooks and Suchey, 1990)	Pelvis	Mean = 38.2 y σ = 10.9 95% range = 26–70
Auricular surface (Lovejoy et al., 1985)	Pelvis	50–59 y
Cementum annulations (Stott et al., 1982)	Upper canine	49.6 y ± 3.6
Cementum annulations (Stott et al., 1982)	Lower canine	48.8 y ± 3.6

The Suchey-Brooks method for estimating age from the pubic symphysis was not possible for the individual with a probable diagnosis of osteogenesis imperfect (S-593 – Saint-Jacques cemetery, France), due to the state of preservation of the ossa pubis. The posterior part of the os coxa is better preserved and the auricular surface of the ilium was examined for age-related changes (Lovejoy et al., 1985). This technique provided an age-at-death range between 40 and 44 years (Table 3). The cementochronology method was based on an upper second premolar and states a higher age at death of 57.6 years ± 1.8 (Fig. 5, c). We had predicted this individual would demonstrate significant discrepancies in age estimates based on the pelvic and cementum annulations and this did turn out to be the case.

Table 3. Estimates of age that obtained for the individual with osteogenesis imperfecta.

Method	**Skeleton part**	**Estimated age**
Auricular surface (Lovejoy et al., 1985)	Auricular surface	40–44 y
Cementum annulations (Stott et al., 1982)	Upper second premolar	57.6 y ± 1.8

Histological analysis also demonstrated indications of dentinogenesis imperfecta (often associated to osteogenesis imperfecta (Teixeira et al., 2008)) (Fig. 6), which was an additional benefit of histological exploration and an offset to the cost of destructive analysis. During the tooth preparation for histology, it was noted that transverse slices of 100um thickness from this individual demonstrated an unusual level of transparency compared to other sections. The dentin also displayed a sparse and irregular tubular pattern under LM examination. The specimen with leprosy demonstrated evidence of root exposure through alveolar resorption and passive eruption; antemortem tooth loss; and apical abscess (Robbins et al., 2009) and thus we hypothesized that cementum annulation formation might be affected by periodontitis or the infectious disease process. MicroCT analysis (LAMIH, University of Valenciennes and Hainaut-Cambresis, France) demonstrated that the cementum was intact and thus histology was performed. We predicted that the cementum would not be intact and could not be assessed for age estimation. This hypothesis was not supported.

Fig. 6. Transverse sections (100 μm) of the mesial third of the root of S-593 (osteogenesis imperfecta) (a) in comparison with another individual preserved in the same conditions (b). The sparse and irregular tubular pattern in S-593 could support the diagnosis of dentinogenesis imperfecta.

For the individual with leprosy (1997–1 – Balathal, India), we predicted cementum annulations would yield significantly discrepant age-at-death estimates compared to those made using the pubic symphysis (Brooks and Suchey, 1990). Age was not estimated from the auricular surface because it was not preserved. The form of the pubic symphysis indicates that this individual was 38.2 years ± 10.9 when he died. Cementum annulation counts based on lower right incisor yielded an age at death of 42.5 years ± 4.0 (Table 4) (Fig. 5, d). Thus age estimates from the pelvis and cementum annulations combined yielded mean estimates of 38–42 years. This result is not consistent with the hypothesis that cementum annulations are unreliable in cases of severe periodontal disease or rhino-maxillary infection. Dental attrition (Brothwell, 1981) yielded an age estimate of 35 ± 5 years, which is consistent with the estimate from the pelvis. Dental attrition somewhat underestimated age-at-death in comparison with cementum annulations but this is not surprising, given severe AMTL and periodontosis, which impacted dental occlusion in the years before death. Cranial sutures provided an imprecise estimate of over 55 years. Cranial stenosis yielded the most discrepant result but this is unlikely due to specific effects of the disease process. This result cannot be explained by this study but cranial stenosis does have a wide margin of error.

Table 4. Estimates of age that obtained for the individual with leprosy.

Method	**Skeleton part**	**Estimated age**
Pubic symphysis (Brooks and Suchey, 1990)	Pelvis	Mean = 38.2 y σ = 10.9 95% range = 26–70
Dental attrition (Brothwell, 1981)	Teeth	35 y ± 5
Ectocranial sutures (Beauthier et al., 2008)	Cranial vault	>55
Cementum annulations (Stott et al., 1982)	Lower right incisor	42.5 y ± 4.0

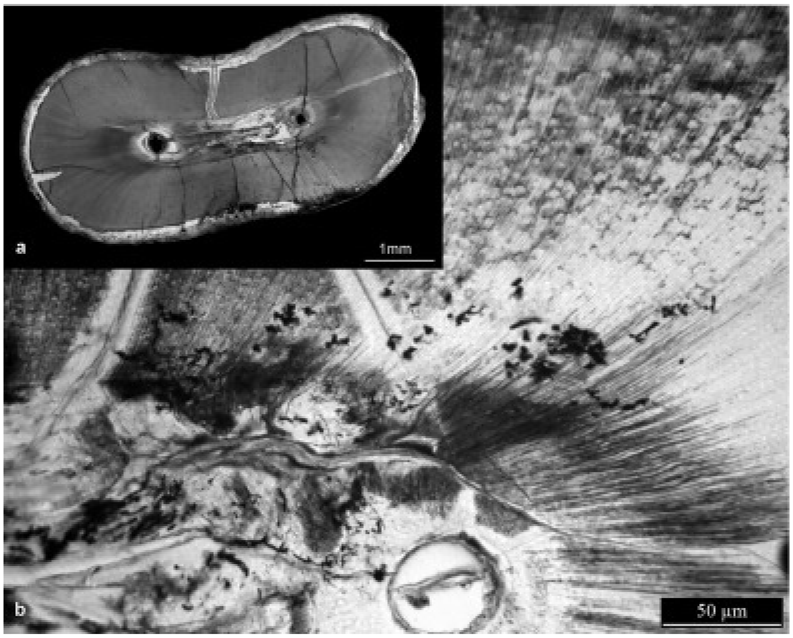

Fig. 7. Transverse sections (100 µm) of the lower right incisor of Balathal showing a dark coloration of dentin (a) and trace evidence of bacterial infection in the dentinal tubules (b).

Histological analysis also revealed trace evidence of bacterial infiltration in the dentinal tubules (Fig. 7). Bacterial penetration of dentinal tubules commonly occurs when dentin is exposed due to an opening of the overlying enamel or cementum (Love and Jenkinson, 2002, Kina et al., 2008). Observation under light microscope shows that bacteria traces are present in the deep dentin proximal to the pulp chamber, as has been demonstrated previously in teeth with apical periodontitis (Peters et al., 2011). Hundreds of bacterial species are known to inhabit the oral cavity but a select group dominates the tubule microflora (Love and Jenkinson, 2002). Therefore, it is unclear from gross examination what species are present. It is also unclear whether the bacterial infiltration resulted from the infection in vivo or if it represents a postmortem process; the latter explanation is initially preferred because the bacteria appear often randomly distributed without regard to tubule orientation. However, mycolic acid analysis may resolve questions about the species represented and how it came to be located inside the tubules. For our purposes in this paper, we found no evidence to suggest the disease process affected the formation of the annulations; they were also apparently unaffected by exposure to the oral environment or taphonomic processes.

4. Discussion and conclusion

Our hypotheses and predictions related to the effect of skeletal growth dysplasias on macroscopic age indicators were supported. The skeletal dysplasias examined in this research were associated with significant discrepancies in osseous age indicators. For the individual with achondroplasia, age-at-death estimated from the 4th rib and dental attrition were significantly different from the estimates obtained by methods based on cranial stenosis and cementochronology. Osteogenesis imperfecta and residual rickets affected the normal progression and timing of morphological changes to articular surfaces in the pelvis, making estimates from these morphological indicators significantly different from the estimates based on cementum annulations.

Our hypothesis that leprosy and periodontosis would affect cementum annulations was not supported. Periodontal disease and leprosy did impact dentin coloration but there was no indication that the formation or preservation of cementum annulations was affected by the disease process or exposure of the root surface to the oral environment. This contradicts earlier suggestions in the literature that leprosy impacts acellular cementum formation (i.e. Ghom, 2005). Rather, compared to the concordance of estimates from the pubic symphysis and the cementum annulations, it appears that the significant amount of antemortem tooth loss may have slightly impacted the effectiveness of age-at-death estimation from dental attrition. Cranial sutures yielded an imprecise estimate of 55+ years, but this difference is not unexpected given the known imprecision of this technique and it is unlikely to be due to the disease process.

The results of this project strongly indicate the need for basic research on the effect of pathology on age estimation in a larger sample, preferably of known-age and health status. Although the techniques employed here for the os coxae, fourth rib, dental attrition, and cranial stenosis are the same techniques in standard usage globally, the discrepancies among them in this limited sample do indicate they cannot be uncritically employed in paleopathological research. We cannot assume that skeletal dysplasias do not affect the accuracy of morphological age estimation techniques. Conversely, we cannot assume that cementum annulations are inaccurate in cases of periodontal and other oral infections. Destructive analysis is not always possible in bioarchaeology and the costs must be weighed prior to undertaking histological research, but cementum annulations may serve as a particularly important tool for age estimation in pathological specimens.

Acknowledgments

The authors thank the editor and the anonymous reviewers for their constructive comments, which helped us to improve the manuscript. Thanks to Sri SR Walimbe and V Mushrif-Tripathy of Deccan College Post-Graduate Research Institute in Pune, India; L. Bernard and P. Demolon of the Direction de l’Archéologie de la Communauté d’Agglomération du Douaisis; and P. Semal of the Institut Royale des Sciences Naturelles de Belgique who granted access to their osteological collections. We would also like to thank S. Vatteoni and W. Devriendt for their help for the retrospective diagnosis.

References

Andersen, J.G., Manchester, K., 1992. The rhinomaxillary syndrome in leprosy: a clinical, radiological and paleopathological study. Int. J. Osteoarchaeol. 2, 121‑129.

Auferheide, A.C., Rodriguez-Martin, C., 1998. Cambridge Encyclopedia of Human Paleopathology. Cambridge University Press, Cambridge.

Beauthier, J.P., Lefevre, P., Werquin, J.P., Quatrehomme, G., Meunier, M., Polet, C., Orban, R., 2008. Les sutures crâniennes ont-elles encore une place dans l'évaluation de l'âge au décès? = Should Cranial Sutures Still Be Considered in the Evaluation of the Age at Death? Journal de Médecine Légale Droit Médical 51, 203‑220.

Bocquet-Appel, J.-P., Masset, C., 1982. Farewell to paleodemography. J. Hum. Evol. 11, 321‑333.

Boldsen, J.L., Milner, G.R., Konigsberg, L.W., Wood, J.W., 2002. Transition analysis: a new method for estimating age from skeletons. In: Paleodemography: Age Distributions from Skeletal Samples. Cambridge University Press, Cambridge, UK, pp. 73‑106.

Brickley, M., Ives, R., 2008. The Bioarchaeology of Metabolic Disease. Academic Press, Oxford.

Brickley, M., Mays, S., Ives, R., 2010. Evaluation and interpretation of residual rickets deformities in adults. Int. J. Osteoarchaeol. 20 (1), 54‑66.

Brooks, S., Suchey, J.M., 1990. Skeletal age determination based on the os pubis: a comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. Hum. Evol. 5, 227‑238.

Brothwell, D.R., 1981. Digging Up Bones: The Excavation, Treatment and Study of Human Skeletal Remains. Cornell University Press, Ithaca.

Cohen Jr., M.M., Walker, G.F., Phillips, C., 1985. A morphometric analysis of the craniofacial configuration in achondroplasia. J. Craniofac. Genet. Dev. Biol. 1, 139‑165.

Deng, C., Wynshaw-Boris, A., Zhou, F., Kuo, A., Leder, P., 1996. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell 84 (6), 911‑921.

DeWitte, S.N., 2010. Age patterns of mortality during the black death in London, A.D. 1349‑1350. J. Archaeol. Sci. 37 (12), 3394‑3400.

DeWitte, S.N., Boulware, J.C., Redfern, R.C., 2013. Medieval monastic mortality: hazard analysis of mortality differences between monastic and non-monastic cemeteries in England. Am. J. Phys. Anthropol. 152, 322‑332.

Doherty, E.S., Lacbawan, F., Hadley, D.W., 2007. Muenke syndrome (FGFR3-related Craniosynostosis): expansion of the phenotype and review of the literature. Am. J. Med. Genet. Pt. A 143A (24), 3204‑3215.

Ghom, A., 2005. Textbook of Oral Medicine. Jaypee Brothers, India.

Hoppa, R.D., Vaupel, J.W., 2002. Paleodemography: Age Distributions from Skeletal Samples, vol. 31. Cambridge University Press, New York.

Iscan, M.Y., Loth, S.R., 1986. Determination of age from the sternal rib in white males: a test of the phase method. J. Forensic Sci. 31, 122‑132.

Kina, J.R., Kina, J., Kina, E.F., Kina, M., Soubhia, A.M., 2008. Presence of bacteria in dentinal tubules. J. Appl. Oral Sci. 16, 205‑208.

Klevezal, G.A., Shishlina, N.I., 2001. Assessment of the season of death of ancient human from cementum annual layers. J. Archaeol. Sci. 28, 481‑486.

Love, R.M., Jenkinson, H.F., 2002. Invasion of dentinal tubules by oral bacteria. Crit. Rev. Oral Biol. Med. 13, 171‑183.

Lovejoy, C.O., Meindl, R.S., Pryzbeck, T.R., Mensforth, R.P., 1985. Chronological metamorphosis of the auricular surface of the ilium: a new method for the determination of adult skeletal age at death. Am. J. Phys. Anthropol. 68, 15‑28.

Maat, G.J.R., 2000. The Impact of Diet on Age at Death Determinations Based on Molar Attrition. In: Forensic Anthropology, Proc. of the Euro. IOFOS Millenium Meeting, Leuven.

Matsushita, T., Wilcox, W.R., Chan, Y.Y., Kawanami, A., Bükülmez, H., Balmes, G., Krejci, P., Mekikian, P.B., Otani, K., Yamaura, I., Warman, M.L., Givol, D., Murakami, S., 2009. FGFR3 promotes synchondrosis closure and fusion of ossification centers through the MAPK pathway. Hum. Mol. Genet. 18 (2), 227‑240.

Milner, G.R., Boldsen, J.L., 2012. Transition analysis: a validation study with known-age modern American skeletons. Am. J. Phys. Anthropol. 148, 98‑110.

Moller-Christensen, V., 1961. Bone Changes in Leprosy. Munksgaard, Copenhagen.

Opperman, L.A., 2000. Cranial sutures as intramembranous bone growth sites. Dev. Dyn. Off. Publ. Am. Assoc. Anatomists 219 (4), 472‑485.

Ortner, D.J., 2003. Identification of Pathological Conditions in Human Skeletal Remains, vol. 1., 2nd ed. Academic Press, London, pp. 645.

Peters, O.A., Bardsley, S., Fong, J., Pandher, G., Divito, E., 2011. Disinfection of root canals with photon-initiated photoacoustic streaming. J. Endod. 37, 1008‑1012.

Pitt, M., 1988. Rickets and osteomalacia. In: Resnick, D., Niwayama, G. (Eds.), Diagnosis of Bone and Joint Disorders, vol. 8, 2nd ed. WB. Saunders, Philadelphia, pp. 2086‑2119.

Polet, C., Orban, R., 2011. L'achondroplase de l'abbaye des Dunes de Coxyde (Belgique, XIIe-XVe siècles). 3ème Colloque International de Pathographie, Paris, De Boccard, pp. 123–162.

Robbins Schug, G., Brandt, E.T., Lukacs, J.R., 2012. Cementum annulations, age estimation, and demographic dynamics in Mid-Holocene foragers of North India. Homo 63, 94–109.

Robbins Schug, G., Gupta, S., Cowgill, L.W., Sciulli, P.W., Blatt, S.H., 2013. Panel regression formulas for estimating stature and body mass from immature human skeletons: a statistical approach without reference to specific age estimates. J. Archaeol. Sci. 40 (7), 3076–3086.

Robbins, G., Tripathy, V.M., Misra, V.N., Mohanty, R.K., Shinde, V.S., Gray, K.M., Schug, M.D., 2009. Ancient skeletal evidence for leprosy in India (2000 B.C.). PLoS ONE 4, e5669.

Robbins, G.R., 2004. Mesolithic Damdama: Dental Histology and Age Estimation. Allahabad University Press, India.

Roberts, C.A., Manchester, K., 2007. The Archaeology of Disease. Cornell Univ. Press, Ithaca, USA.

Roberts, C.A., 2002. The Past and Present of Leprosy: Archaeological, Historical, and Palaeopathological and Clinical Approaches. Hadrian Books, Oxford, UK.

Roksandic, M., Vlak, D., Schillaci, M.A., Voicu, D., 2009. Technical note: applicability of tooth cementum annulation to an archaeological population. Am. J. Phys. Anthropol. 140, 583–588.

Schmitt, A., 2002. Estimation de l'âge au décès des sujets adultes à partir du squelette: des raisons d'espérer. Bulletins et mémoires de la Société d'Anthropologie de Paris 14 (1–2), 51–73.

Séguy, I., Buchet, L., 2011. Manuel de Paléodémographie. Ined, Paris.

Stott, G.G., Sis, R.F., Levy, B.M., 1982. Cemental annulation as an age criterion in forensic dentistry. J. Dent. Res. 61, 814–817.

Teixeira, C.S., Santos Felippe, M.C., Tadeu Felippe, W., Silva-Sousa, Y.T., Sousa-Neto, M.D., 2008. The role of dentists in diagnosing osteogenesis imperfecta in patients with dentinogenesis imperfecta. J. Am. Dent. Assoc. 139, 906–914, quiz 994.

Wittwer-Backofen, U., Gampe, J., Vaupel, J.W., 2004. Tooth cementum annulation for age estimation: results from a large known-age validation study. Am. J. Phys. Anthropol. 123, 119–129.

Wood, J.W., Milner, G.R., Harpending, H.C., Weiss, K.M., 1992. The osteological paradox. Curr. Anthropol. 33, 343–370.