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Review

The problem of aging human remains and living individuals: A review

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ABSTRACT

Forensic anthropology is affected by the unavoidable limits concerning difficulties in standardization of methods and procedures; age estimation is one of the main tasks of forensic anthropology and odontology, both on the dead and the living: literature has shown several methods of age estimation, and although they may be thought of as equivalent, every procedure has its limits, mean error, practical situation and age range where it gives the best results; the lack of standardization and consensus concerning which method can be used, as well as the lack of a practical approach in different cases is the main limit in a correct age estimation process.

This review aims at exposing the experience of the authors working in the FASE (Forensic Anthropology Society of Europe) subsection of IALM (International Academy of Legal Medicine) in the field of age estimation both on the dead and the living, at highlighting advantages and limits of each method, and suggesting practical solutions concerning the age estimation process for adults and subadults, dead and living, and pedopornographic material.

Contents

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1. Introduction

It is a clear fact that one of the main problems in forensic anthropology (FA), as in many other forensic disciplines, is the lack of "consensus" or uniformity of procedures and methods used. In particular there are specific subareas of FA which need constant updating and harmonization, as, for example, aging, PMI (post-mortem interval determination), determination of ancestry, etc. So far, very few of such recommendations have been produced. One of the main goals of associations of forensic anthropology such as FASE, a subsection of IALM (International Academy of Legal Medicine) [1,2] to whom the authors belong as founding members or members of the Board, is to fill this gap, by bringing together scientists with forensic experience (this working group in particular has a cumulative experience across Europe of over 2000 cases of unidentified human remains, as well as numerous cases of living people where age estimation has a judicial importance) and try to produce a thorough review and practical recommendations on specific issues such as aging.

Aging in the forensic context is necessary both for the dead and the living. For the dead it is principally to aid identification in creating a biological profile which can then be compared to missing persons. For the living the aim is to solve judicial or civil problems concerning age of minors as regards questions of adoption, imputability, pedopornography and, for adults, civil issues on pensionable age and other similar matters for individuals lacking valid identification documents. It should always be borne in mind that, whatever the case is, all a forensic anthropologist or odontologist can do is give the best estimate of biological age…regardless of how far it may be from actual chronological age, provided these limits are made clear to judicial authorities.

Some reviews on aging have been performed in the past. There are several age reviews, for instance, in the archaeological context [3,4]. However these may not be exhaustive for forensic purposes because the goals are different and the human material may be different (different states of preservation, taphonomic effects, etc.); also judicial requirements and time are another important factor. There cannot be a simple transferral of methods from the historical disciplines to the forensic context.

Some efforts were performed for the forensic scenario: the main one in 2000 [5] (which, 8 years after, obviously needs updating, although there has not been a great progression in new methodologies). In other words age at death estimation continues to be the Achilles' tendon of anthropology. More recently Rosing et al. [6] and Schmeling et al. [7,8] published recommendations for forensic diagnosis of age on skeletons. However this review does not seem to cover all forensic scenarios and gives no clear cut practical suggestions.

Finally, there is *The Study group on Forensic Age Diagnostic*, a German group, which recently produced articles [7,8] concerning aging the living in the forensic scenario. Although useful general indications are given, the authors do not refer to specific methods for practical conditions.

Both for the living and the dead, the only age one can try to achieve is physiological age which might be quite different from chronological age. The older the person is, the larger is the discrepancy between physiological age and chronological age. Therefore the older the person is, the less accurate the methods are, as can be seen with the increasing error range. Also, different methods are examined with different statistical procedures which make results incomparable.

Additional problems can be the reference samples on which the various methods have been developed, which are also of paramount concern since the methodologies become too specific or dependant on the demographical and chronological profile of the series [9,10].

Research has produced several articles, many of which still have not been tested on different populations, or in different taphonomical contexts, for example. So the operator may feel lost in front of a large quantity of methods available. For this reason even at the risk of seeming excessively simple, the authors have tried to give operators practical suggestions based on literature and experience.

Literature has provided, throughout the past years, several methods aimed at determining age; the most commonly mentioned in the forensic scenario and most acclaimed in literature can be divided into dental and skeletal methods. Among the most popular methods for teeth in case of age estimation of dead individuals one should mention Logan and Kronfeld [11], and Schour and Massler charts [12,13], with revision by Anderson et al. [14] and Ubelaker [15]; cementum annulation by Kagerer and Grupe [16]; the Lamendin method [17,18], and in case of age estimation of the living, the Hunt and Gleiser method [19], Gustafson and Koch [20], Demirjian et al. [21] and its revisited version by Willems et al. [22], Moorres et al. [23], Liliequist and Lundberg [24], Nolla [25], Haavikko [26], the Harris and Nortje method [27], Kohler et al. [28], Kullman et al. [29], Mincer et al. on the third permanent molar [30,31] and aspartic acid racemization [32–34]. The most common skeletal procedures in cases of age estimation of dead individuals are summarized in textbooks such as those by Fazekas and Kosa [35,36] and Scheuer and Black [37]; one should also mention diaphyseal length from long bone measurements [38,39]; cranial suture obliteration as indicated by Meindl and Lovejoy [40], Masset [41], Nemeskeri et al. [42] and Baker [43], pubic symphysis evaluation by Todd [44,45] and Suchey–Brooks [46,47], chondral articular surface of IVth rib analysis by Iscan, Loth and Wright [48–50], ilium auricular surface observation by Lovejoy et al. [51], microscopic analysis of bone structure and osteon counts by the Kerley method [52], improved in the Kerley and Ubelaker revision [53] and revisited in the Ahlqvist and Damsten method [54], Stout and Paine [55]; in cases of age estimation in the living, evaluation of clavicle sternal end fusion degree [56–58], analysis of ossification and fusion of wrist and hand bones by Greulich and Pyle [59], the Tanner–Whitehouse technique [60] and the FELS method [61] are usually mentioned.

However, without knowledge and experience of these and other methods, it is difficult to find one's way in a real case. The following wishes to be a practical guide through the more appropriate and user-friendly methods for forensic purposes, and provide suggestions for cases which in the forensic scenario are just beginning to make their way, such as requests for aging adults in view of pensionable age. It is clear, as mentioned previously [5], that for a method to be considered applicable, it must follow specific requirements: (1) the method must have been presented to the scientific community, as a rule by publication in peer-reviewed journals, (2) clear information concerning accuracy of age estimation by the method should be available, (3) the methods need to be sufficiently accurate, and (4) in cases of age estimation in living individuals special principles of medical ethics and legal regulations have to be considered [8]. Nonetheless, sometimes the best methods are not those with the best published standard error, but those which have been tested by many on different and numerous populations, which are suitable for a specific forensic scenario, practical, user-firendly, relatively quick and cheap.

2. Aging the dead

2.1. Subadults

Classically subadults are divided as follows: fetuses; newborns; infants (0–6 years); children [10–15]; adolescents [19–27]. For these cases the subdivision of the methods was not performed according to body parts or state of preservation but for practical reasons it seemed more convenient to work according to age group (as one will see later, adults will be divided according to state of preservation). Though it may seem an incoherent mode of reasoning, experience has shown that this is the only practical approach for the subadult field. Also, body preservation is irrelevant in most cases since all methods (radiographic if soft tissues are still present or macroscopic examination for clean skeletal remains) are based on the same rationale: dental mineralization and eruption; presence of ossification centers; dimensions of bones; epiphyseal fusion.

Literature has pointed out that age estimation based on dental methods is more reliable than from skeletal analysis; in detail, dental methods are supposed to be less influenced by racial and environmental factors. Nevertheless, having a reference population to which the sample belongs to is of the utmost importance: even in case of subadults in particular, where the error range is more limited, correct age assessment requires that the most accurate reference population be chosen along with the most appropriate method.

2.1.1. Fetuses (Table 1)

In general, dentition is more reliable than skeletal development, so the rationale should be to first (if possible) verify dental development, then skeletal. If crowns are present inside the crypts X-rays should be performed and the Schour and Massler [13] scheme, processed by Logan and Kronfeld [11], is still one of the easiest to follow amongst the dental methods, despite some disadvantages, such as the narrow age ranges and lack of information concerning variation by tooth.

From a skeletal point of view, and although it dates to almost 30 years ago, data given by Fazekas and Kosa continue to be largely used [35,36]. Even recommendations by Scheuer and Black are based on it [37]. Recent literature is attempting to validate the methods with updated radiographic data [62].

2.1.2. Newborns (Table 2)

In order to verify whether the fetus is full term one must check the following elements: the greater fontanelle has to be open and the smaller ones should be in the process of closing or closed; the ossification of the distal epiphysis of the femur should be present; mineralization of the tip of the cusp of the first permanent molar visible in X-ray. Diaphyseal length of long bones is not determinant (because of variation related to body size) but one should measure tibia, femur or humerus as a general indicator [63]. A summary of such methods as shown by Scheuer and Black [37], and Ubelaker [15] can be extremely useful.

Table 1
Recommendations concerning age estimation methods for dead fetuses; standard errors go from 0.5 to 2 years.

	Recommended approach*	References
Fetuses	Dental development	[11–13]
	Presence of ossification nuclei	[35,36]
	Long bone development	[37]

* Most methods suffer from sex and ethnic bias

Table 2
Recommendations concerning age estimation methods for dead newborns; standard errors go from 0.5 to 2 years.

	Recommended approach*	References
Newborns	Dental development	[11–13]
	Diaphyseal length of long bones	[37,63]
	Presence of ossification nuclei	[35,36]
	Check also mineralization of cuspid of first permanent molar and ossification of femur distal epiphysis	

* Most methods suffer from sex and ethnic bias

Table 3
Recommendations concerning age estimation methods for dead infants and children; standard errors go from 0.5 to 2 years.

	Recommended approach*	References
Infants and children	Dental development	[12,13,15,21,64]
	Diaphyseal length of long bones	[37–39]

* Most methods suffer from sex and ethnic bias

2.1.3. Infants and children (Table 3)

Tooth calcification and eruption are the methods of choice, which have been calibrated extensively on mandibular and maxillary teeth. Several tables and charts exist. The Schour and Massler charts [12], Demirjian's method [21] (frequently used for the living) and Haavikko [26] on the first molar are recommendable though Demirjian [21] has proven to overestimate age both in males and females. Moreover, the Demirjian method shows a lower inter-operator variability, especially on third molar development assessment thanks to its clearly defined stages [65–67]. However, low accuracy may arise in case of application on different ethnic groups (with respect to the original French Canadian sample). Developing tooth length has proved to be an accurate method to estimate age as well. Liversidge and Molleson [68] proposed regression equations which are easy to apply. Skeletal development (as mentioned for the previous sections) should also be assessed: a comparison between dental and skeletal age is always necessary in order to corroborate results (and verify possible discrepancies) and also to look for indication of sex: in fact males have a skeletal delay in growth with respect to females.

For infants, in the skeleton one must check for the ossification of skull bones, mainly temporal, occipital and frontal bone and the mandible. In children, for long bones, one should consider long bone diaphysis length and appearance of ossification centers and the fusion of epiphyses [69–73]. In these cases, the age estimation will be better performed by a comparison with tables from the correct reference population in order to reduce impact from racial and environmental factors.

2.1.4. Adolescents (Table 4)

The same general rules apply as for children; however more specific and accurate information can be obtained by applying methods which were devised for aging the living. For example, age estimation in adolescents, infants and children can be assessed by evaluation of skeletal development of hand and wrist bones, by X-ray examination and comparison with the Greulich and Pyle atlas [59], the Tanner–Whitehouse score [60,74] and the FELS method [61]. The quickest and sufficiently accurate method for this purpose is the Greulich and Pyle atlas. Interesting indicators of the beginning of puberty are the fusion of the hook of the hamate and

Table 4
Recommendations concerning age estimation methods for dead adolescents and subjects in Transition phase; standard errors go from 0.5 to 2 years.

	Recommended approach*	References
Adolescents	Dental development (and Demirjian)	[12,13,15,21,64]
	Long bone development	[37,76–78]
	Maturation of hand and wrist (Greulich and Pyle, Tanner–Whitehouse)	[59,60]
Transition	Third molar development (Mincer)	[30,31]
	Fusion of spheno-occipital/basilar synchondrosis, iliac crest and vertebral ring	[37,76–78,80]
	Maturation of hand and wrist (Greulich and Pyle, Tanner–Whitehouse)	[59,60]
	Fusion of clavicle sternal end	[37,57,58]

* Most methods suffer from sex and ethnic bias

capping of the epiphyses of the third metacarpal. This technique is useful in age estimation in the living as well because X-ray exposure is confined to a limited anatomical area and the number of bones examined is high, although literature has pointed out that results provided by dental methods are sometimes more reliable than evaluation from methods based on skeletal growth [73–75] (see Section 6.1).

On the other hand, as a very general indication, the end of adolescence could be checked through the fusion of thoracic and lumbar vertebral rings [76,77], and iliac crest with X-ray examination, through the Risser staging [78,79]. These methods however should be used only when other body parts are not available.

2.2. Transition phase (Table 4)

There are some dental and skeletal elements which demarcate the end of growth—this means that they are fairly good specific markers for restricted age ranges which go from adolescence to early adulthood (between 20 and 25 years), where in some cases methods for aging the living are recommended (see appropriate section).

These are: 3rd molar development evaluation according to Mincer et al. [30,31]; obliteration of the spheno-occipital or basilar synchondrosis [80,81];[1] fusion of the sternal end of the clavicle [56–58] and hand and wrist development. This will indicate if one should go on to apply "Adult" methods or go back to "Adolescents".

2.3. Adults

Preliminary remarks: In a single forensic case, the goal of age estimation is to reconstruct the biological profile in order to reach positive identification through a comparison with the biological data from missing persons. The method used and error range may be crucial in a match with missing persons. For example, if the method gives a 30–45 age range and the "right" missing person is 47, this method will tend to exclude that person. Moreover, lack of accuracy can lead to incorrectly exclude the target individual. In all forensic reports to investigators, results must be given with a large age range (minimum 20 years). Nevertheless, a more restricted age range could be given in the report only to start the investigation in case of missing persons (e.g.: age estimation 20–40 years, but start the investigation with individuals around 25–35). Therefore the results of age estimation need to be standardized according to the specific case.

Age estimation is more difficult in adults than in subadults because skeletal and dental development have already settled, with a consequent increase of error range; the main logic at assessing age in adults is based on the physiological degeneration observed in skeletal and dental structures with age. The mechanical shock absorber function provided by articular cartilage requires the absence of district vascularization; the fragility of articular structures with respect to mechanical stress and difficulties of adequate cartilage feeding support an early degeneration with age. However, articular degeneration is modified by pathological and occupational factors, which may radically modify the degeneration process, overcoming the importance of aging. The same limits are observed in dental wear, where physiological and pathological elements both contribute to dental structure degeneration with aging.

Recently a new means of estimating age of adult skeletons has been proposed by Milner et al. [82]. The authors argue that transition analysis has forensic significance because it allows one to investigate age throughout the entire lifespan, moreover it can be used for partial or complete data sets [82].

Taking into account the innumerous number of methods and the different states of preservation of bodies, in order to be practical, our recommendations have been divided according to body preservation: the first group is that of well preserved unidentified bodies, visually recognisable, but which remain unclaimed and unidentified, the second group will be called "decomposed bodies": it includes putrefied, mummified, saponified and burnt bodies, and comprises all states of preservation which severely alter physiognomy. The third group comprises skeletonised bodies; the fourth calcined remains; the fifth body parts (with indications according to each single part).

2.3.1. Fresh well-preserved body (Table 5)

In these cases apparent age can be given, which however is unscientific and subjective. The two-step procedure (TSP) [10,83] in general is a good choice in all cases. This consists of combining the pubic symphysis Suchey–Brooks [46,47] system with the dental Lamendin method [18]. For SB phases I, II, III the age estimate is given using the chronological interval corresponding to each phase. If the SB phase is 4, 5 or 6 the Lamendin method is applied. In case of lack of pubic symphysis, the 4th rib (Iscan) (48–50) can be used (first 4 stages, age by Iscan, last 4 stages age by Lamendin). Lamendin is considered by literature one of the most accurate methods of age estimation for over 40-year-old dead subjects [84], but in the later years, especially in case of periodontal disease, the method underestimates the real age with a 19-year mean error [85]; this method however can be applied very easily at the autopsy room in a few minutes. Furthermore, recently Ubelaker and Parra [86] have shown that there is minimal impact of population variation, which increases the reliability of the method in estimation of age at death across various populations.

In order to apply TSP, at autopsy the pubic symphysis or 4th rib must be removed, macerated, cleaned and then read. When the fourth rib is not available, the third or fifth ribs can be used [87]. In order to remove the pubic symphysis correctly, the normal xifosinfiseal cut is performed, the soft tissues in front of the pubic symphysis are manually detached and then, once one has identified ischio-pubic and ileopubic *rami*, a section should be performed across the two *rami*, in order not to cut too close to the symphyseal surface and damage it (for maceration one should leave the symphysis immersed in water and detergent, adding a biological cleansing agent every second day).

If there are no teeth at all available or if the sample has been interred for a long period, in substitution of Lamendin, osteon count analysis is a good option (by Kerley–Ubelaker, Ahlqvist–Damsten or Stout and Paine methods [52–55]). They are based on a microscopical analysis of a bone section from long bone diaphyses in order to perform an osteon count. The Ahlqvist–Damsten

[1] Shirley and Jantz [81] proved it was more useful as an age indicator in males and that its utility in females is questionable.

Table 5
Recommendations concerning age estimation methods in case of fresh, decomposed, skeletonised and calcined remains; standards errors go between 7 and 15 years.

	Methods	Literature	Remarks
Fresh	Suchey–Brooks	[46,47]	More reliable between 20 and 40 years
	Iscan	[48–50]	Higher reliability after 60 years
	Lamendin (in TSP protocol)	[17,18,83]	Periodontal disease influence; high reliability between 40 and 60 years
	Microscopic analysis of osteons	[53]	More time-consuming
Decomposed	As in well preserved case, according to the single case		
Skeletonized	Suchey–Brooks	[46,47]	High reliability between 20 and 40 years
	Iscan	[48–50]	High reliability after 60 years
	Lamendin (in TSP protocol)	[17,18,83]	Periodontal disease influence, high reliability between 40 and 60 years
	Auricular surface	[51]	Inter-individual and intra-individual variations
Calcined remains	Microscopic analysis of osteons	[53]	With great caution, part of the specific bone of origin the fragment comes from must be known and not on calcined material

method considers variation of bone structure in the subperiosteal area, but results have proven to be less reliable than data obtained from the Kerley–Ubelaker technique [53,88]. In these cases there is no need to perform decalcified sections; undecalcified sections (quick and cheap) are easily obtained [89].

In the future, the possibility of applying CT scans should be considered since they allow one to evaluate areas (such as pubic symphysis and auricular surface) which are difficult to reach anatomically in less time: applicability however still need to be tested.

2.3.2. Decomposed (Table 5)

In these cases, not even apparent age can be given, therefore biological methods are even more important. To such bodies one should adopt the same procedures as in the previous section.

X-rays should be performed on these bodies to check for the presence of dental or osseous elements which may still be in formation or fusion (see Section 3).

2.3.3. Skeletonised (Table 5)

In the case of a complete skeleton, a variety of methods can be applied. One should keep in mind that all anatomical areas presenting lesions should be excluded from age estimation. Since the skeleton has all parts useful for aging exposed (unlike the case of the entire body), the dilemma concerns using one preferred method or many methods with a multifactorial approach. Though literature is still unclear in relation to this issue [9,83,90], our experience allows us to suggest the use of two methods. If possible, the two-step procedure, once again, can give optimal results. If teeth are missing or the pubic symphysis and ribs are damaged then some methods should be avoided, such as suture closure; auricular surface may be a better option. Concerning auricular surface (Mendl–Lovejoy–Mensforth method [51]), though reliable, intraobserver error is significant, leading sometimes to inaccurate results. However the method is still useful in a forensic context particularly if associated with evaluation of the acetabulum, though this latter method is younger and still needs more testing [91–93]. The acetabulum, in the future, may in the end be the most reliable for 60-year-old or older individuals.

In all these cases a general recommendation is that if the pubic symphysis or rib give indications of belonging to a very young adult, then it is advisable to confirm the state of epiphyseal union of the sternal end of the clavicle (see Section 3).

2.3.4. Calcined remains (Table 5)

In these cases (frequent examples are remains in burnt cars) obviously the choice of the method depends on the preservation of anatomical sites and how badly burnt they are. None of the dental methods for adults should be used for aging burnt remains apart from perhaps pulp chamber methods [95–98], which, however, still need further testing. X-rays should always be performed on burnt bodies to check for dental and osseous development, in case the person is an adolescent or subadult. If the remains consist only of burnt bones, a possible method could be osteon counts [53,54], since heat may not significantly alter osteon shape or disposition for these purposes [94]. However one should use such methods cautiously for several reasons. First of all, most methods require that the anatomical site of the bone be identifiable—obviously sometimes in cases of badly burnt bone this is not possible, therefore the method should be avoided. Secondly some bones are so badly calcined that the histological structure can no longer be read and interpreted—in these cases also osteon counting cannot be performed. As regards the more important issue of bone shrinkage, several authors have investigated the matter. Bradtmiller and Buikstra, indeed, reported that bone shrinkage induced by the effect of burning does not appear to have a significant effect on age estimation [94]. In 1990, Hummel and Schutkowski [99], despite the fact that cremation made the distinction of the histological structures more difficult, found acceptable results for histological age estimation of cremated human remains. However, they caution that if the bones are too fragmented, it may not be possible to find the specific locations on the bones that are required for each method [99].

In his study, Nelson [103] commented on the results of Bradtmiller and Buikstra and found that it was premature to minimize the effect shrinkage may have. He concluded that more research is needed. In 2005, Thompson [100,101] found alterations of the size of the bones and of the microscopic porosity in burned bones. He concluded that his results "suggest that anthropological techniques applied to burned bone will likely be detrimentally affected and accuracy will be reduced". However in his study, he did not tackle the histological structure as Nelson had. So it is not possible to conclude from this study that histological techniques for age estimation cannot be used in cases of burnt bones. Nonetheless much caution should be applied if no other method is applicable, taking care to exclude from such studies calcined bone.

2.3.5. Body parts (Table 6)

If age estimation must be applied to a skull with teeth, suture closure and dental wear should be used only as general indicators (broad orientation) and never as accurate methods. Tooth wear is dependant on too many variables such as attrition, diet and occlusion; loss of teeth may depend on social habits, such as drugs, or infections and diseases (not necessarily will an edentulous mouth belong to an old individual although it is more probable); suture closure [40–43] is increasingly problematic since there is much inter-individual variability and methods which have been devised have extremely large intervals/standard deviations for forensic purposes: this method, supposed to have been developed for the first time by Vesalio [102], shows a higher reliability with

Table 6
Recommendations concerning age estimation methods in case of body parts; standards errors go between 7 and 15 years.

	Methods	Literature	Remarks
Skull with teeth	Lamendin	[17,18,83]	Periodontal disease influence, high reliability between 40 and 60 years; not tested on heated samples
	Carbon-14 analysis	[108,109]	Useful only with date of birth after 1950; still being studied
	Aspartic acid racemization	[32–34]	Further studies are required cross-laboratory and cannot be used on burnt samples; questionable on buried and decomposed samples
	Cranial suture	[40–43]	Too wide inter-individual and intra-individual variability
	Pulp chamber/tooth ratio (Kvaal and Cameriere)	[95–98]	Great caution: still need further studies and calibrating
Skull without teeth	Cranial suture	[40–43]	Too wide inter-individual and intra-individual variability
	Aspartic acid racemization	[111,112]	Further studies are required cross-laboratory; difficult use on bone due to remodelling
	Osteon analysis	[110]	High inter-individual variability; large error
Postcranium without skull	Suchey–Brooks	[46,47]	High reliability between 20 and 40 years
	Iscan	[48–50]	High reliability after 60 years
	Auricular surface	[51]	Inter-individual and intra-individual variations
Torso	Iscan	[48–50]	High reliability after 60 years
Upper limbs	Acsadi–Nemeskeri (humeral trabecular rarefaction)	[116]	Outdated method, high racial and sex variability
Lower limbs	Microscopic analysis of osteons	[53]	More time-consuming

maxillary sutures [104], but is affected by a wide inter and intra-individual variability [105]: moreover, sutures in the same individual may show different degrees of fusion.

When all teeth are present, the use of Lamendin – if PMI can be reasonably pinpointed to a recent period – is recommended. In fact Lamendin et al. [18] has not been tested on samples which have been buried in soil for several decades and must be used with caution on historical samples [106]. It could therefore be implemented with pulp chamber methods (Kvaal and Cameriere), which consider the ratio between pulp chamber and total dental area in upper and lower canines or other teeth [95–98], and which have greater possibilities of not being affected by taphonomy. These methods are preferable to cementum annulation [16] because the latter is more time-consuming, expensive, not user-friendly and less accurate. Recently, its applicability has also been seriously questioned [107]. If no monoradicular teeth are present, the methods available remain other pulp chamber methods [98], dental wear, cementum annulation and aminoacid racemization. Problems associated with dental wear and cementum annulation have been mentioned above; concerning aminoacid racemization, though it seems to be more precise and with a smaller error with respect to all other methods [32–34], it should be kept in mind that very few labs in the world perform this technique and have prepared calibration curves for this purpose, that it is much more expensive and that it has only been tested on recent material: for certain it cannot be applied to burnt material and it is not known what effects water, soil and time may have.

In 2005, Spalding et al. [108], and in 2006, Ubelaker et al. [109] pointed out radioisotope concentration analysis in dental enamel; the increase of carbon-14 by thermonuclear devices between 1950 and 1963 (captured in biological structures which undergo very little remodelling in life, such as primary dentin and enamel) allows one to verify if childhood and consequent tooth development occurred before that time span, with consequent extrapolation of the time of birth [108,109]. An advantage of this method is that the period in which a person was born is determined within 1.6 years. Yet, for individuals born before 1943 (12 years before the onset of nuclear bomb testing) this method allows one only to say that birth occurred before that year [109]. The application of this approach is less limited in the case of soft tissues, because of the faster trend in carbon-14 substitution in biological structure and the eventual environmental contamination. Such a method is interesting but obviously more expensive and needs further testing.

In case of skulls with no teeth, obviously, if all teeth have been lost antemortem it is more likely that the person be old (although as mentioned above one cannot exclude the presence of a younger adult). With the skull only, one is left with very inaccurate possibilities: skull suture closure (ecto [41] and endocranial); osteon count and aminoacid racemization on bone. Problems with sutures have been already mentioned above; for osteon counts on the skull, one publication has used the occipital bone [110] and the authors themselves do not recommend this method for age estimation; aminoacid racemization on bone, on the other hand, is much less accurate than on teeth because of bone remodelling, and therefore, cannot be recommended as a reliable method for forensic purposes [111,112].

In cases of the entire postcranium without the skull the pubic symphysis is recommended. If the result is within the first 3 stages of Suchey–Brooks, then leave it at that. If it belongs to the last three stages, then a method which is more accurate and precise for older individuals such as osteon analysis should be used.[2] One can always confirm age estimation by pubic symphysis with the 4th rib and auricular surface. Looking at the acetabulum also is convenient, with the limits mentioned previously.

If only the torso is available, Iscan's rib method is recommended; Iscan's method has proved to be the most reliable especially after the age of 60 and in females; in males in fact recent literature has shown a morphological variability between surfaces of different ribs [113] while Yoder et al. [87] has shown that the method could be used on the third to the fifth ribs (histological morphometry on ribs still needs further testing [114,115]). In the case of only upper limbs, examination and assessment of humeral bone structure by the Acsadi and Nemeskeri combined method [116] are recommended. If age estimation must be applied to the lower limbs osteon count with adequate equations by Kerley–Ubelaker method should be preferred. Once again, aspartic acid racemization has been proposed on bones [111,112] in literature; however the limits have already been mentioned above.

Finally, methods involving molecular biology (and DNA) are being assessed but are at their very beginning [117–119].

[2] Some authors have already started defining and testing the need for new Suchey–Brooks phases including older individuals [122].

Table 7
Recommendations concerning age estimation methods in the living.

	Methods	Literature	Remarks
Subadults	Physical assessment and Tanner sexual classification	[129–131,135]	High racial and inter-individual variability; strong criticism concerning its use for forensic purposes
	Bone development (radiological): Greulich and Pyle atlas and Tanner–Whitehouse	[59,60]	Greulich and Pyle user-friendly, quick, some population data available
	Dental develompent (radiological): Demirjian method	[12,13,15,21,64]	Some population data available
	Dental development (radiological): Mincer method	[30,31]	Asset: gives prrobability of having reached 18 years; disadvantage: high frequency of third molar agenesia (up to 8% in Caucasoids)
	Clavicle sternal end fusion (radiological)	[37,57,58]	Still being tested
Adults	Physical assessment and hormonal dosage for women	[136]	High racial and inter-individual variability
	Pulp chamber based methods	[95–98]	High error range (Kvaal–Solheim method); need for further experimental data (Cameriere method)

In conclusion, as regards aging adult human remains, it should be mentioned that there are at the present virtually no methods for the estimation of age at death of the elderly. In other words it would be virtually impossible to reasonably discriminate between 70 and 80 or 90.

A summary of recommended methods, including literature references, are shown in Tables 1–6.

Therefore, aging dead adults is still riddled with limits, large errors [120,121], and population specific references are needed [9]. Nonetheless, with appropriate knowledge of the limits, a safe age indication can be given to investigating authorities.

No single skeletal indicator of age at death is ever likely to reflect accurately the many factors that accumulate with chronological age. In fact, one must use as many dental and skeletal indicators as possible. However, in order to maximize the potential of each method, in the final evaluation one should consider mainly the method or methods that have a lower inaccuracy for a particular age range, once one has placed the individual in a general young/old age group by preliminary selection with the pubic symphysis or the rib [90].

3. Aging the living (Table 7)

In the last years cases of age estimation in the living have become more and more frequent. The main issues of age estimation in the living concern adoption, imputability (14,16,18,21 years depending on the country), and old age pension (50, 55, 60, 65 years, again depending on the country).

With respect to the dead and the relative requirements for a biological profile, aging the living requires (1) the use of non invasive methods and (2) a higher accuracy and precision because of specific legal requests.

Criteria for age estimation in the living have been given recently by *The Study Group on Forensic Age Diagnostic* [7,8], with special attention to sensitive legal and ethical implications; the group has proposed guidelines for age estimation in the living, with a three-step procedure including a physical examination and anthropometrical analysis, sexual development assessment (clinical/medical examination is first of all important to diagnose retardation, disease and syndromes which could influence the estimation of skeletal or dental maturation), dental analysis by orthopantomogram, and X-ray study of left hand; if the 21-year threshold is considered, clavicle sternal end X-ray examination [123] is suggested (newer studies suggests that collar bone assessment is essential already at the age of 18). Although this approach is one of the few attempts at standardizing the procedures of age estimation, no recommendation concerning the single method and in detail the specific field of application of each method is given. In our opinion some information on specific methods available and their limits may result more useful though this inevitably implies more frequent updating.

A correct assessment of age estimation in the living should consider therefore (1) a physical examination, (2) bone development, and (3) dental development.

3.1. Physical examination

For adoption and imputability purposes (and all other age assessments for non adults or young adults) one should perform a physical examination in order to verify growth and particularly sexual development, although the limits and cautions of this assessment need to be taken into consideration.

The first evaluation should consider a physical observation and collection of the medical information from the proband through a complete anamnesis. First, height and weight must be accurately measured and the measurements should be compared with the specific percentiles concerning the standard growth of children [124,125] provided by WHO and CDC. These charts however are commonly used in clinical practise, and therefore should only be applied in order to obtain a general orientation for forensic purposes; in addition, physiological and pathological factors, as well as the social and economic contexts have proven to influence body development [126–128,134]. The same can be said for the assessment of sexual development; pediatricians commonly use the Tanner stages, based on the analysis of sexual traits in males and females [129,130]. In detail, the Tanner method considers in females breast and pubic hair development, and in males increase in testicular volume and pubic hair. This method is used in the clinical context. However, important limits concerning racial and inter-individual variability have been pointed out [131–134], and Tanner himself has stressed that the method should not be used for chronological age and thus forensic purposes [135].

For adults, although body and sexual growth have terminated, a complete physical examination with perhaps clinical tests, should not be excluded; in females in fact menopause accompanies physical modifications and can be confirmed by hormonal dosage [136] and this may be useful as a general indication—although menopause also is affected by ethnic and inter-individual variability.

3.2. Skeletal and dental assessment of children and young adults (Table 3)

3.2.1. Skeletal development

After the physical examination, one should then go on to perform a skeletal assessment. In older days, a total body X-ray was performed, but this has been proven redundant and a useless radiation exposure in view of the alternative method, i.e. the performance of an X-ray on the hand–wrist area (generally the left hand, but when the person is left-handed, the right hand may be preferable).

Many methods exist to evaluate the growth advancement of the hand–wrist region, and in detail among the others the most popular are the Greulich and Pyle atlas [59], Tanner–Whitehouse [60], and FELS [61] methods. The Sempè method [137] and other methods have nowadays, particularly for forensic purposes, been supplanted by the GPA and TW methods. Even the more recent FELS method is rarely used. This computerised method provides an evaluation concerning concordance between the wrist and hand X-ray pattern and the age declared [61]. It is however more complex and less user-friendly than the other two methods.

The Greulich and Pyle atlas (GPA) [59] provides a series of X-ray standards of the hand and wrist from birth to 17/18 years for females and 19 years for males; the method is a comparative one and consists in the evaluation between the X-ray examination from the proband and the standards from the atlas. The final result includes mean age estimation and an error range. The Tanner–Whitehouse (TW) method is based on a scoring system which evaluates the ossification degree and morphological appearance of the ossification nuclei and bones in hand and wrist [60]: a maturity score is then given, with the indication of mean age and error range.

The first question which arises is which to use among the two. Literature exists in favour of either: Some authors [138,139] refer that the GPA method gives similar reproducible results, is faster, and user-friendly. Others prefer the Tanner–Whitehouse method [140–142] and seem to give less intraobserver error, particularly for TW2 [141], although one study [74] refers that TW2 should not be used whereas TW3 can be considered appropriate. There seems to be a general majoritarian consensus that the two methods reliably assess age, though GPA is quicker and more user-friendly, and that the main issue is that of actually testing the method used on the population the proband belongs to [74,143]. The fact that in literature slightly more cross-population studies have been performed for GP than for TW perhaps indicates an implicit preference for the first.

As mentioned previously, many factors influence dental and skeletal development—pathological (some well known clinically visible diseases which affect growth), socioeconomical status [144] and also infections such as HIV [145]. Another main problem is ethnicity. The issue of the applicability of the methods to different populations has been questioned by many. However up to now no large scale studies have been performed. A major study has recently been done on racial differences in growth patterns of children assessed on the basis of bone age [146]. In this recent important review, the authors perform GPA on 1390 male and female African, Asian, white and Hispanic subjects ranging from 1 day to 18 years. Results showed that bone age was significantly overestimated in Asian and Hispanic children, who seemed to mature sooner than their African American and white peers. Therefore ethnic and racial differences in growth patterns exist at specific ages, and the GPA method at times may not recognize this fact [146,147]. This is why population data is extremely important, and for the GPA method such data is beginning to appear: Danish [148], Moroccan [149], Spanish [150], Malawi [151], Thai [152], Turkish [153], Dutch [154], Pakistani [155], Chinese [156,157], Swedish [158], Asian [159], Italian [160,161], Austrian [162], German [163], and many others [164–168]. These articles begin to show, according to the case, the straightforward applicability or the need to standardize the method according to the specific population—an issue of utmost importance.

Similar studies have been performed for the TW method on North American [169], Austrian [162], English [170,171], Brazilian [172], Korean [173], Chinese [174–177], Japanese [178–180,188], Belgian [181], Danish [182], Mexican [183], Indian [184], Portuguese [185], Australian [186], even Hottentot populations [187], with similar conclusions.

In countries where the age of 21 years may be of some legal interest, fusion degree of clavicle sternal end may provide relevant information because it occurs in the age range useful for imputability assessment [56–58]; moreover, use of NMR, although the first results have yet to be confirmed, may overcome limits in utilizing X-ray examination [57].

3.2.2. Dental development

Among the numerous dental methods which exist for aging children, adolescents and young adults, Demirjian and Mincer (for the third molar) are the most commonly suggested by literature; Demirjian is based on the growth assessment of several teeth (usually half a dental arch). Though considered a reliable method, several studies have shown the high dependance on the specific characteristics of the population; after the first studies on a French Canadian population [189], the method was used on different populations, showing relatively high differences between the chronological age and estimated age [190–211], with a general overestimation in all the studies, in some cases amounting to 1 year [198]: in detail, different studies have pointed out that the method needs a standardization specific for every population. The Demirjian method has been applied so far on different populations, and in detail French Canadian by the author [189], Finnish [190–192], Chinese [193,205], Norwegian [194], British [66,195], German [196], Indian [198,199], Australian [200], Turkish [204], Hungarian [201], Polish [203], Malays [206], Korean [208] and Senegalese ones [202], with a general wide or slight overestimation. The comparison of results with the original population the proband belongs to is therefore indispensable. However, the well-defined stages and objective evaluation makes Demirjan's method one of the most suitable for forensic purposes. Moreover, where the original method was applied with specific calibration curves, the results showed an increase in accuracy [197]; this confirms that, although the morphological stages are well defined and easy to use, the high ethnic variability requires particular caution in discussion of the final result.

In a recent congress organized by FASE, an experimental study showed that the best methods as regards bias (average difference) and median absolute difference between the estimated and chronological age were Willems' (with a bias of 0.12 years and a difference of 0.52 years) and Liversidge's flat distribution (with a bias of 0.03 years and a difference of 0.69 years) in a sample of 946 children [212]; although literature suggests the use of classical methods, other methods should not be disregarded: moreover, who deals with age estimation in the living should be brought up to date on the development of known methods and new methods provided by literature, in order to use the most adequate technique and to more precisely discuss the results.

The Mincer et al. [30] method also is a good option for older adolescents where the third molar can give relevant information. This method also has begun to be tested on various populations. In detail, it was proven on an American [30], American Blacks [213], American Hispanic [214], German [215], Japanese [216,217], African [218,219], Austrian [220], Turkish [221] and Spanish population [222]; a high variability in age threshold of every Mincer stage has been verified with different mean ages. Pathological factors have not been considered so far, but they should be among the exclusion criteria of subjects from the studies; this means that the dental age estimation methods should be applied to subjects who have a healthy dental profile. The lack of information concerning the possible alteration of dental development by pathologies, which is better known in the case of skeletal development, is a relevant limit which must be overcome. In the few articles where pathologies were not an exclusion criterion, no particular alteration in dental development was observed [218]: on the other hand, subjects with severe alteration in dental development sometimes were not affected by specific pathologies. However, the scanty literature concerning this topic prevents us from drawing any definite conclusion.

The importance of ethnic variation in the assessment of dental development requires further population studies in order to reach an adjustment of each method according to the specific population, with an increase in precision and accuracy. In this scenario, the higher interest of anthropological and forensic journals for population studies (one example is the new Forensic Anthropology Population Data section of Forensic Science International) will help by providing new data useful in the discussion of the final result, and in the evaluation of the methods themselves.

One particular issue which should be kept in mind for court purposes is the fact that what interests judges is whether these individuals have reached a specific threshold; explanation of the error is therefore crucial. Most of these methods give standard errors or standard deviations, therefore the response to a judge will be, for example, 17.5–18.5 years. Which may put him or her in a "difficult" situation, in which it would be helpful to know the probability of that person actually having reached the threshold (e.g. 18 years). In this way judges know what they are risking. And in this perspective, methods such as the Mincer one, which along with the mean age and error give the probability of having reached the limit of 18 years, could be more useful to the judge. In this perspective a study [223] was performed on 47 judicial cases: these cases all involved immigrants with no valid documents which were the object of medicolegal expertises since it was not known whether they were of age or not (18 years old). All skeletal methods and dental ones gave borderline results, which would have made the decision difficult for the judges. An examination of the motivations of the final sentences showed that when the Mincer method was applied, giving the probability of the individual having reached the age of 18, the judges expressed appreciation for the fact that in this way, the risk they were taking (for example of sending a juvenile to jail) was easier to comprehend. Interestingly, it was seen that when the probability of the individual of being 18 or over was over 70%, the judges felt "confident" in taking the risk of an "adult" verdict.

3.3. An ethical note: the X-ray assessment for forensic purposes

Finally, one should mention the issue of performing X-rays on the living for legal purposes. Established doses for X-ray examinations in forensic age estimations are low and vary from less than 0.1 μSv (left hand X-ray) to up to more than 800 μSv (CT). Computed tomography on children is – however – a relatively high-dose procedure. The comparison between risks associated with radiation and other common risks from daily hazards shows that – despite the higher sensibility of children to radiation – hand radiographs and OPGs are harmless. The responsible use of X-rays in forensic age estimations, however, demands a critical selection of methods that are suitable for the specific age range. From this point of view, the use of collar bone CT is not appropriate for the age estimation of children under the age of 18, and is only indicated as a method for answering the question of whether the proband is over 18/21 years. Despite the foregoing discussion of all the difficulties in the actual assessment of risk, the data show that it is indispensable not only to use dose-lowering techniques, but also to take the age of the person being examined into greater account [224–227].

A solution to the X-ray exposure for age estimation may derive from use of ultrasonography and NMR technology [171,228]: the use of ultrasonography in detail has been mainly aimed at the assessment of ossification of clavicular sternal extremity [229]; moreover, an ultrasonographic version of the Greulich and Pyle atlas was developed (with a high correlation with the original radiographic atlas version), which has so far shown a 6-month error increase in 84.4% of males and 88.5% of females under 6 years [230]. However, at the moment, the use of ultrasonography and NMR technique for forensic purposes needs adequate standardization, though in the future these methods may offer more precise and safe indications.

3.4. Age estimation in juvenile pornography images

Finally, another application of age estimation methods on the living (young) concerns the growing plague of pedopornography, which more often requires an age assessment of represented subjects from photos; the crime of pedopornography is based on the specific ages each country considers as relevant for this crime (usually 14, 16 or 18 years depending on the country). In these cases judges require from the expert witnesses age estimation of individuals represented in photos or videos. The most relevant limits in this type of analysis are the poor quality of the images, the application of clinical and anthropological methods on 2D pictures, make-up and shaving of represented subjects.

A study aiming at verifying the unreliability of age assessment based only on body characteristics has proven that the sexual characters on pictures are often deceiving [231]. This study has suggested that the use of the assessment of sexual characteristics for forensic purposes has a limited importance—as Tanner himself has stressed. This study has proven the need for a new approach for age estimation from 2D pictures; a research field which may hold some answers could be the morphological and metrical analysis of the face, in order to verify a relation with chronological age [232–240]: however, at the present time, the scanty number of experimental studies which have been so far carried out prevent us from considering this technique as a valid option. In videos in which individuals smile or open their mouth, it may be possible to evaluate dental eruption and development, which may give a rough idea of the age range.

Thus, in general, physical and sexual maturation stages should be used with great caution for forensic purposes, particularly on photographic material.

3.5. Adults

In these cases very few methods can be used: age assessment is more difficult in adults than in subadults in the living as well, and at the present there is no recommendation concerning the most appropriate method to use. Root methods (Kvaal, Cameriere) may provide a useful tool in age estimation in adults, if future literature will offer further population data confirming their reliability [95–98]. The above-mentioned amino acid racemization on dentine also has been suggested, however relevant limits are met because of the fact that it involves destruction or damage of the individual's tooth—which may bring on ethical problems. A general assessment of physical status (with hormonal dosage for women [136]) and the use of dental methods at the moment is all scientific literature has to offer.

4. Conclusions

The authors have shown the main problems in age estimation and suggest some practical solutions for adult and subadult aging both dead and living; different approaches should be taken for different body preservation stages (for the dead) and for different age ranges. Furthermore, one must realize, for the dead, and particularly for adults, that large age ranges are the rule—and at the moment there is no way out. Although some methods may be tempting because novel and bearing low errors, for forensic purposes it is safer, when one can, to use the more traditional and standardized methods, possibly tested on different populations, and apply the correct population reference for the individual one is studying.

Another question concerns processing information from different methods. The simple pooling of data cannot be performed, because every method expresses its error range in different ways (mean error, standard deviation, etc.): some methods, such as the Lovejoy technique, do not provide an error indication. Interpolating results from different methods, however, is possible provided a rough age indication is given. This can be performed by Principal Component Analysis (PCA), which has proven to be reliable [90].

The consequent issue concerns how to present results to investigating authorities; an age estimation report must include indications about the methods performed, information about specific limits, and the mode of processing data.

Finally, it is necessary to point out that age estimation concerns biology, where variability is the rule; even considering population data, every individual may show different aging patterns. A correct age estimation must consider this unavoidable limit, in order to define with precision the limits of results provided.

The present review therefore has the intention of practically guiding the forensic pathologist or anthropologist dealing with aging through the enormous literature on this issue – perhaps one of the most frequent topics of anthropological publications – which at times may be misleading and confusing.

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