Chapter 4

Biological vs Legal Age of Living Individuals

Francesco Introna and Carlo P. Campobasso

Summary

The demand of forensic age determination in living individuals deals mostly with juvenile or subadults because in most European countries, the legally relevant age limit ranges between the 14th and 21st year of life. Among the variety of scientific procedures available in age assessment, there is wide agreement that methods based on sexual, skeletal, and dental maturity are suitable. Unfortunately, there are obviously problems with using “age” in a system measuring maturity and therefore, biological age (sexual, dental, or skeletal) does not always correspond to chronological age (legal). None of the modern techniques is both easy to use and practical, as most of them result in a slight over- or underestimation, depending on the applied method, the range of human variation between sex and population, and discrepancies between biological and legal age. The margin of error can be substantial in living individuals, sometimes by as much 2–3 yr either side, or at best, 12 mo, even by combined methods. The physician should use all the available evidence, including multiple maturity indicators, and must approach each case with caution, because the poor correlation between maturity and chronological age represents a fundamental limitation to age-determination practices in forensic settings.

Key Words: Age determination; living individual; skeletal and dental maturity.

1. INTRODUCTION

Age estimation of living individuals is one of the most difficult problems in clinical forensic medicine. It is a prerequisite for personal identification, and

From *Forensic Anthropology and Medicine:
Complementary Sciences From Recovery to Cause of Death*
Edited by: A. Schmitt, E. Cunha, and J. Pinheiro © Humana Press Inc., Totowa, NJ

it is increasingly important in criminal matters. In fact, if doubts arise regarding the age of a person suspected of a crime (i.e., robbery, extortion, fraud, physical assault, arms offences, murder, etc.), forensic age estimation is promptly requested by authorities to ascertain whether the person concerned has reached the age of imputability (criminal responsibility). In most European countries, the legally relevant age limit range is between the 14th and 21st year of life (*1*). For example, in Italy as well as in France and Germany, the relevant age limit above which a person has legal responsibility is 14 yr, and the age limit of 18 is decisive for establishing whether juvenile delinquency law or general criminal law in force for adults is to be applied.

In Europe, the demand of forensic age determination deals mostly with juvenile or subadult delinquents, but it also deals with illegal young immigrants and refugee children requesting political asylum. The need for accurate techniques for age estimation has never been greater than in last two decades because of the increase in international migration because of recurrent armed conflicts, which has led to a steady rise of foreign populations in many European countries. Most young immigrants have no valid proof of birth date (legal age); they lack valid identification documents; they also either do not know their age or are suspected of not giving their correct age. For asylum seekers, the refugee's birth might never have been registered or identity documents never issued. In such individuals, age estimation is therefore crucial, and represents one of the main requests made by police and judicial authorities to physicians.

The request may also come from a child's legal representative or from a government agency. In the context of international protection, the United Nations High Commissioner for Refugees (UNHCR) remarks in relation to refugee children that it is often necessary for the asylum country to determine the age of a young person who has, or is claiming, refugee status, because there should be different procedures or programs for refugees who are below a specific age, for example, 14 or 18 yr (*2*). In such a case, the UNHCR states that children seeking asylum should not be held in detention (section 7.6 of UNHCR guidance), and that children should be given the benefit of the doubt if the exact age is uncertain (section 5.11c of UNHCR guidance). Therefore, when identity documents are not relied on to establish age, authorities usually need not only age calculation mainly for differentiating the juvenile from the adult status, but also in relation to school attendance, social benefits, adoption procedures, employment, and marriage.

For this purpose, physicians such as pediatricians, dentists, or forensic experts are often asked to give their opinion on whether a person is younger or older than 18 yr. The indicators most commonly used in assessing subadult

age include somatic, sexual, skeletal, and dental maturity. Among the variety of scientific procedures available in age assessment, there is wide agreement that the following methods are suitable: physical inspection with determination of body measures and signs of sexual maturation, radiographic examination of the left hand and wrist, and examination of the oral cavity by a dentist with evaluation of an orthopantogram (3).

1.1. Physical Inspection

The clinical examination of the living individual is the first step of the aging process, as the visual recognition of somatic maturity is the simplest age estimation method. Pediatricians usually base age assessments in children on constitutional type and physical appearance of sexual maturity indicators.

Physicians commonly use height and weight measurements as primary tools for assessing a child's physical growth in relation to other children of the same gender and age. These anthropometric measures are noninvasive, inexpensive, easy to perform, and are also well related to the general increase in size and length of the whole body (4). During the physical examination, they are commonly recorded with stadiometers and properly calibrated scales. Based on stature and weight, body mass index (BMI) is also easy to obtain because it is defined as weight in kilograms divided by the square of stature (standing height) in meters. BMI was only recently recommended as additional routine measurement of growth, as it is a valuable index of body composition (adiposity) to determine underweight or obesity in the clinical environment by comparison of an individual to age and sex-specific percentiles from a reference population (5).

During the inspection of maturity indicators, the development of an individual's secondary sex characteristics (sexual age) is also used in the age-assessment process, especially at puberty (usually between the ages of 8 and 13 yr; the average age is 11 yr). The appearance of pubic and axillary hair, for example, can reflect progressive stages in the sexual maturation of boys (with the concomitant growth of external genitalia such as penis and scrotum) and girls (with the concomitant breast enlargement and onset of menstruation). Breast, genital, and pubic hair development can be classified for boys and girls, using Tanner's five stages (6,7), which is a sequential process that children go through as they pass into puberty. For example, the onset of puberty in boys is marked by testicular enlargement, and pubic hair usually appears initially on the scrotum and at the base of the penis developing to the adult stage, with a final distribution covering the pubes and the medial surface of the thighs (Table 1). In girls, the first menstrual period can occur during the fourth or fifth Tanner stage, usually at around 12 or 13 yr of age. Once a girl

Table 1
Tanner Staging System for Males

Stage	Pubic hair	Penis	Testicles
I	Preadolescent. No pubic hair (except for a fine peach fuzz).	Penis is in the same proportions as in childhood.	Testicles are in the same proportions as in childhood.
II	Sparse growth of long, slightly darkened, pubic hair at the base of the penis.	Slight enlargement.	Testicles begin to get larger. The scrotum begins to get a reddened and roughened texture.
III	Pubic hair increases its pigmentation and gets coarser and curlier, spreading sparsely over the pubic bone.	Increased length more than width	Increased size with further scrotal enlargement. The scrotum texture becomes more like that of an adult.
IV	Hairs have adult characteristics, but not adult distribution. They cover the base of the penis and begin to grown on the upper part of the scrotum.	Further penile enlargement and increased breadth. The glans, or head, of the penis becomes much more prominent.	Further enlargement. The scrotal skin gets darker in color.
V	Adult distribution with spread to medial thighs.	Adult size and shape.	Adult size and shape.

has reached Tanner stage 5, her breasts are fully formed and her pubic hair is adult in quantity and type, forming the classical upside-down triangle shape common to women (Table 2).

However, the sequence and timing of the particular events that occur during puberty (especially if compared with progressive rise in stature) vary considerably from one person to the next because of differences among sex, populations, and socioeconomic factors (8). The onset of puberty is also extremely variable based on sex: girls may have the first signs of pubertal changes at about the age of 8 or 9 yr and boys at about 9 or 10 yr. Equally, pubertal delay can also take place, and the first signs may be significantly delayed to 14 or 15 yr of age, especially in boys.

Ethnicity and socioeconomic factors (mainly environment and diet) may affect the onset of puberty. Alterations in nutritional status and illness can delay the pubertal changes so that a person may actually be older than the

Table 2
Tanner Staging System for Females

Stage	Pubic hair	Breasts
I	Preadolescent. No pubic hair except for a fine, peach fuzz body hair.	A small, elevated nipple with no significant underlying breast tissue.
II	Sparse growth of long, slightly darkened, downy straight hair, mostly along the labia.	Breast bud. There is elevation of the breast and nipple (papilla) as a small mound with increased areolar diameter.
III	Pubic hair increases its pigmentation and gets coarser and curlier, spreading sparsely over the mons veneris area.	Further enlargement and elevation of the breast and areola (with no separation of their contours), which begins to darken in color.
IV	The hair grows in more densely. Hairs have adult characteristics, but not adult distribution.	Projection of the areola and nipple (papilla) to form secondary mound.
V	Adult distribution with spread to medial thighs.	Nipple elevated, areola contour continuous with breast.

physical appearance leads one to believe. Some nutritional deficiency diseases (such as rickets or iron-deficiency anemia), infectious diseases (such as osteomyelitis, smallpox, brucellosis, and so forth) and congenital disorders (such as Turner's syndrome, which is a gonadal agenesis, or achondroplasia or osteochondrodysplasias, which are abnormalities of cartilage and/or bone development) give rise to disproportionate growth (9). Therefore, it is not possible to give a precise age of an individual based only on physical examination or clinical experience. In the clinical setting, measurement of growth is crucial for detecting and treating illness or disorders of development, not for age estimation.

Several growth charts for height and weight (10), standardized percentile curves of BMI for children and adolescents (11), or charts showing the range of ages during which each pubertal event occurs (12) are available to compare an individual's development pattern with that of a healthy standard group of the same age and sex. These comparisons measure the body's general level of maturation, providing only little information of the individual's age and very poor quantitative data on the accuracy of such assessment. In fact, most growth charts are expressed in centile lines rather than standard deviation scores (13).

Percentile curves represent the distribution of the measurement of interest (e.g., height, weight, BMI) and so, what percentage of children of the same gender and age show the identical pattern of pubertal changes. For example, the 50th percentile line represents the mean value for age, so that 50% of children will be above this point, and 50% will be below it. A child of average height and average timing of pubertal growth spurt would follow the 50th percentile throughout life. Based on this way of expressing a child's developmental level of maturity, pediatricians can determine the percentile cutoff points (e.g., 3rd or 5th, and 95th or 97th centile lines) useful to identify children that are too tall or too short, underweight or overweight, compared with children of the same gender and age from reference normal populations.

Therefore, the physical examination usually gives only a glimpse or, even better, an empirical probability on the chronological age of the individual. It can be useful in adolescence or before 14 yr, but not so much in subadults. In fact, most of the above-mentioned physical events related to puberty have already occurred before the critical age limit of 18 yr, when the request of forensic age determination is usually submitted. Specifically around 18 yr, other different indicators of maturity have been studied to assess an individual's age level, among which dental growth and skeletal development are the most commonly used in young adults. Like bones, teeth progress through maturational stages, undergoing continuous change in size and shape. The developmental changes in bones and teeth are intimately related to those of the reproductive system, which is directly responsible for most of the body's externally discernible changes. Therefore, bones and teeth greatly reflect the functional status of the reproductive system and provide a useful index of the general growth during the adolescent period (14).

The assessment of dental and/or bone maturity by X-rays represents the most objective method among the range of diagnostic procedures available for age estimation, and appears to be also suitable for forensic application in living individuals. Evaluation of ortopantomograms and X-rays of the left hand and wrist is the method of choice widely used by experts for forensic age assessment. Radiographic examination of other segments of the skeleton provides only little additional information to the aging process; it is also not recommended because it involves an unnecessary increase in radiation exposure to the rest of the body (1).

1.2. Dental Age

Dental growth and emergence (tooth eruption) have long been recognized as the most useful criteria for estimating age because dental age can be analyzed both radiologically and through clinical examination. Studying tooth

emergence by clinical inspection of the oral cavity is simple and does not require expensive tools or equipment. Younger ages can be assessed visually with greater accuracy because more teeth are undergoing formation, and the intervals between morphological stages are shorter, and therefore, more precise (15). However, all permanent teeth except the third molars have finished their development after the median ages of 15 or 16 yr. In fact, if the third molar crowns are less than half complete, it is virtually certain that the subject is less than 14 yr old (16). Late in adolescence, only the third molars continue to form (17). At this stage, the third molars have completed their crown developments, and the roots are the only dental structure that can be used for age estimation (18) up to a median age of about 19–20 yr, when apex closure has taken place (19). In younger adults, the calcification stages of the third molars are the most reliable dental indicators, but in living individuals, they can be examined only by X-rays or ortopantomographs (Fig. 1).

Gleiser and Hunt (20) first suggested that an assessment of tooth calcification, rather than emergence, may be more useful for age estimation. Actually, the worldwide reference method of age determination in living individuals remains the Demirjian's system, which classifies eight stages of crown and root formation used to score the third molar development (21). This system is based on a 20-yr study of more than 7000 subjects of French-Canadian origin, aged 2–20 yr. Using Demirjian's eight-grade classification, a study of the American Board of Forensic Odontology (ABFO) reported adequate probabilities of age assessment based on the level of third molar formation, providing regression formulae relative to the medicolegal question of whether an individual is at least 18 yr of age (17). If the third molar root apices are completely closed, and the periodontal ligament has attained a uniform width (stage H of Demirjian's system), there is a high probability (90.1% for white males and 92.2% for white females) that an individual is at least 18 (16). The ABFO study also observed that according to Kullman et al. (18) and Levesque et al. (19), maxillary third molars formation was slightly advanced over mandibular third molars, and root formation occurred earlier in males than females. Recent papers have confirmed such findings with respect to the earlier development of third molars in males compared with females, and the same trend of an earlier third molar development in the maxilla compared with the mandible (22–24).

However, several limitations of the Demirjian's system have been recently illustrated, among which the most important are the following: it is based on observations derived from radiographs, the choice of the tooth developmental stage is quite subjective, and age cannot be precisely evaluated after 16 yr (25). Specifically around 18 yr, the different analyses regarding the wisdom

Fig. 1. Orthopantomographs at age of 13 yr, 6 mo (A) and at age 17 yr, 10 mo (B).

teeth do not yield precise conclusions (22,26,27). In this matter, the ABFO study also remarks that the third molar is a nonideal developmental marker because it is the most variable tooth in the dentition with regard to size, time of formation, and time of eruption, and it can be congenitally absent, malformed, impacted, or extracted (17).

Thorson and Hagg (26) already suggested that the dental development of the third mandibular molar should not be used for estimation of chronological age in individuals because of its very low accuracy. Based on accuracy tests, the authors stated that the 95% confidence interval of the difference between estimated age and true chronological age is quite large, about ± 4.5 yr

in girls and ± 2.8 yr in boys. A more recent article also provides a confidence interval age estimate up 95%, and regression formulae with a standard deviation of 1.49 or 1.50 yr for males and females, respectively (24), which is quite a short difference between age prediction and chronological age. However, it is obvious that most of the studies on wisdom teeth calculate only probabilities for an individual to be above or below a predefined age, such as 18 yr. Actually, the 100% confidence in dental age assessment has been never reached, and no one can make an exact judgment or a certain prediction of age. There is only an empirical probability of an individual being at least 18 as based on stage of third molar formation (16). It is important to note that even before the age of 18, some or all third molars might have reached complete root development (21,28). This indicates, according to Mesotten et al. (22), that linking the completion of root development (closure of apical ends) of one or more third molars to an age over 18 yr is unsatisfactory.

Such remarks should be kept in mind by all experts and investigators involved in forensic age estimation. In the scientific literature, there is quite absolutely unanimity that, based on dental growth, it is impossible to determine exactly whether a young person is younger or older than 18 yr. The result of age estimation is an extrapolation by a probability procedure. Therefore, it is impossible to give a precise age; the only appropriate way is to give an age interval for a specific confidence (29).

A possible option to increase the precision and the accuracy in the dental age-estimation process by root development of the lower third molar could be to use an additional stage of root mineralization in the Demirjian's system (18), or comparing the standard deviation with other skeletal age calculation techniques based on, e.g., hand and wrist bones. In fact, according to Ubelaker (30), it is generally agreed that assessment of age reflects greater accuracy when derived from multiple indicators. The investigator should always use multiple sources when attempting age estimation based on the assessment of dental and skeletal maturity, as they offer superior results to those obtained by single age indicators (31), especially in living individuals.

An extensive literature review of forensic odontology concluded that teeth are better suited for age estimation than bones (29,32). Developing teeth remain a widely used method to assess dental maturity, and the formative stage of the third molar can be certainly considered the only quantitative biological variable available for estimating the age of an individual in his late teens or early 20s (17). However, some authors still assume that skeletal methods are more accurate (33).

1.3. Skeletal Age

The growth of the human skeleton is of major importance for the aging process as the appearance of ossification centers and union of epiphysis relate to a fairly definite sequence and time table that makes skeletal maturity a reliable age indicator according to sex and ethnical differences. For the first three decades of postnatal life, age changes in the bones of the upper and lower extremities are relatively very reliable and may give age estimates within 1–2 yr (14).

Determination of skeletal development is clinically relevant as it provides the only means of assessing rates of maturational change throughout the growing period (34). Skeletal maturity assessment is a common procedure in pediatric radiology, and is frequently requested as part of the evaluation of children who are either too tall or too short for their chronological age. Such assessment can also be useful in the management of children with various endocrinopathies (especially those involving the pituitary gland, thyroid, and gonads) or in children with malformation syndromes, and in planning orthopedic procedures in which the outcome may be influenced by subsequent growth of the child (e.g., surgical management of scoliosis or leg-length discrepancy). In many cases, the decision whether to treat a child with growth hormones depends on the outcome of bone age estimation (35).

One of the best known methods of measuring skeletal maturity in children and young adults is by radiological assessment of hand and wrist bone development. This is a medical procedure often used in forensic settings to provide evidence about a person's chronological age by measuring skeletal maturity. X-rays of the left hand and wrist are usually taken, and their use is strongly recommended by experts because they show enough detail for age estimation (Fig. 2). The hand and wrist region has a large number of ossification centers and can be easily radiographed with minimal radiation exposure to the rest of the body. In fact, an X-ray of the hand and wrist involves a very low dosage of radiation and presents negligible risk of contamination (8).

The Radiographic Atlas of Skeletal Development of the Hand and Wrist made by Greulich and Pyle (G&P) in 1959 (36) is the most commonly used bone age standards. The atlas method has the advantage that it is quick and easy to use. A given individual's hand radiograph is compared with the standard films in the atlas, and the closest match is obtained. A well-trained radiologist takes few minutes to determine the bone age from a single hand radiograph.

Standards such as those of G&P were designed to provide clinicians and others with clear definitions of average development, but they do not provide data, however, on the possible range of variation around the mean (30,37).

Fig. 2. Hand–wrist X-rays at age 13 yr, 2 mo (**A**) and at age 18 yr, 3 mo (**B**).

Unfortunately, this method has also some other limitations, as it is less objective and quite imprecise, especially if applied to a modern or different ethnic group. In fact, the atlas was compiled solely from Caucasian children of the upper socioeconomic class who lived in the 1930s. Some authors have demonstrated that the G&P standards must be used with reservations to determine bone age in children of today and of diverse ethnicity, particularly when making clinical decisions requiring accurate skeletal age in black and Hispanic girls and in Asian and Hispanic boys in late childhood and adolescence ([38,39](#)). Another recent study found the G&P standards also imprecise for American children of European and African descent born after 1980, concluding that new standards are needed to make clinical decisions that require reliable bone ages and accurately represent a multi-ethnic pediatric population ([40](#)).

Following the G&P atlas, several other authors proposed more sophisticated systems for predicting age from carpal and wrist bone appearance and

development. A valuable alternative to the G&P atlas method is based on assigning numerical scores to bones, depending on their levels of maturity. Since 1975, Tanner et al. (41) improved and extended Acheson's method (42), developing the Tanner and Whitehouse system (TW2), which makes use of eight or nine stages for each of 20 hand and wrist bones. However, because of the presence of many metacarpal and phalangeal bones in the hand and wrist X-ray, the authors promulgated two more separate scores for skeletal maturity, one concerning the carpals only and the other the radius, ulna, and short finger bones (RUS).

The method TW2-RUS seems to be more reliable than the G&P method (43,44), because the standards for bone age derive from research based on a large sample of approx 3000 modern boys and girls. A large-scale comparison of the atlas-matching method of G&P and the bone-by-bone approach of TW2-RUS (45) measured greater intraobserver variation for the G&P method than for the TW2 (95% confidence limit, -2.46 to 2.18 vs -1.41 to 1.43 , respectively). Furthermore, because the two methods of bone age assessment did not give equivalent estimates, Bull et al. (45) suggested that one method only (preferably the TW2) should be used.

The TW2 method seems to be more accurate than the G&P atlas system, but it is also a more rigorous and time-consuming approach. An average time of 7.9 min for TW2 and 1.4 min for G&P assessments has been calculated (46). Furthermore, problems in assigning bone ages by the TW2 method arise from many sources (47), among which the most significant is poor positioning of the hand when the radiograph is taken. This error can alter the radiographic appearance of the epiphysis and makes interpretation unnecessarily difficult.

In particular, several investigators have commented on the variance between radiography and macroscopy in the evaluation of the progress of epiphyseal union (48). In fact, most papers illustrating the development of the centers of ossification are based on radiographic survey data, rather than dry bone. However, because the process of epiphyseal fusion begins in the center and can progress quite far before the external epiphyseal margin is fused, sometimes the epiphysis can radiographically appear completely fused, whereas macroscopically it is only partially fused. Thus, the X-ray would indicate a more advanced stage of union than would the naked eye. Some authors suggest that the radiograph might show union as much as 3 yr earlier than is anatomically apparent (49), but Krogman and Iscan (50) argue that the difference is no more than plus or minus 6 mo.

On the other hand, during the process of fusion there is a buildup of compact bone at the epiphyseal plate. This can appear on X-ray as a line of

radiodensity, sometimes referred to as a “persistent line of fusion,” which remains visible up to several years after the epiphyseal line appears macroscopically to be completely obliterated (14). In such a case, if this radiodense line is interpreted as evidence of recent fusion, the X-ray might indicate a less advanced stage of union than would be osteologically apparent. It is therefore clear that, because currently both macroscopically and radiographically based standards are used in anthropology and medicine to estimate skeletal age, the forensic expert should avoid using standards developed on osteological basis to interpret radiological observations (48).

A concomitant problem in the application of radiographic standards for epiphyseal union is the lack of uniformity in evaluating stages of union. This is also because of the severe limitations in comparability, reproducibility, and verification of the different methods recommended by several scientific groups, each of them using their own protocol. Other sources of error in clinical practice are the lack of consistency in repeat ratings of the same film by one or more observers (intra- and interobserver error) and using the system outside the limits of its design (47). Computerized systems for assessing skeletal maturity have been developed with the aim of reducing many of the inconsistencies associated with radiographic investigations. Computerized bone age estimation has the obvious advantage of saving the radiologist's time because the computer, not the operator, usually rates the bone. The image is digitalized and then represented by a large number of mathematical coefficients. Furthermore, computer estimates are likely to be more reproducible than human estimates because of the automatic recognition of different stages of bone maturation. Based on the TW2-RUS method, the so-called computer-assisted skeletal age scores (CASAS) have been created to increase reliability and validity (51,52). CASAS seems to be more reliable and more valid estimator of skeletal maturity than the manual version of the TW2-RUS method (53).

Among the computer-aided methods to assign skeletal age, worth mentioning is that one developed recently by Niemejer et al. (54), but the most common is certainly the Fels method designed by Roche et al. (55), which uses 98 maturity indicators. Using the Fels method, the radiologist rates the bone, and the recorded grades are entered into a computer that provides the bone age and the standard error. This is the only method, to the best of the authors' knowledge, that provides an estimate of error (possible range of variation around the mean), very useful in forensic settings. Unfortunately, experience with such computer-aided methods (i.e., CASAS) as well with the Fels method is very limited, and the most commonly used methods are still the G&P atlas and the manual version of the TW2-RUS. However, because the assessments made using the G&P atlas are often inaccurate (56), at present, the manual

version of the TW2-RUS should be considered the golden standard in forensic settings and the most reliable method of age estimation, in accordance with the considerable changes recently made by the authors (57).

Several medical experts have criticized the reliance on age testing by hand and wrist X-rays and emphasized the inaccuracy of this practice (58). For example, based on a comparison between the TW2 and Fels methods, van Lenthe et al. (59) have found no agreement in skeletal ages. Such discrepancies can be ascribed not only to differences in the statistical methods of the scoring system and the scales of maturity, but also to differences in maturation of the reference population. In their atlas, G&P (36), explicitly recognized the discrepancies in chronological and skeletal ages and numerous studies of the bone age standards—such as those based on dental development—have found similar discrepancies. In fact, even if dental and skeletal development have strong relationships with chronological age, the accuracy of this relationship is greatly affected by many factors, among which are sexual and population variability, and also genetic, nutritional, metabolic, environmental, or functional factors. Therefore, skeletal age does not necessarily correspond to chronological age.

2. BIOLOGICAL VS CHRONOLOGICAL AGE

In this matter, the concept of biological age, as opposed to a chronological age, needs to be introduced. According to Baer (60), biological age represents “an aggregate or composite of many discrete development factors,” most of them intimately related with each other. It is not feasible to assess all the systems in the body, so most commonly, only certain key indicators of development are used (8), such as somatic, sexual, dental, or skeletal maturity indicators.

Based on the methodological approaches already mentioned, age estimation of a living individual involves establishing the physiological age of the skeleton, constitutional and sexual type, tooth eruption and/or mineralization, and then attempting to correlate them with chronological age. The assumption for age assessment is that sexual, dental, and bone development is unanimously considered representative of the general physical maturity. Unfortunately, chronological age, sexual age, dental age, and skeletal (or bone) age are not necessarily the same in a given individual. Deviation among these four ages is common and well appreciated in medical practice because biological age (sexual, dental, or skeletal) does not always correspond to chronological (legal) age. The main reason for such discrepancies is that assessments of an individual’s age measure maturity, not chronological age, which is age measured by the time (years and months).

There are obviously problems with using “age” in a system measuring maturity. A sexual, skeletal, or dental year, unlike a chronological year, is not constant at all stages of development, and maturation levels can differ within and between individuals (8). There is an intimate relationship between chronological age and biological age indicators, but it is neither constant nor linear (61). In fact, a number of physical traits are expressed in terms of age equivalents, and by comparing these, one can determine whether a child is advanced in some areas and slow in others.

For example, the bone age represents an equivalent level of skeletal development rather than a true chronological age (62). Bone age is used to determine skeletal maturity for assessing biological development in clinical and auxological studies. Hand and wrist X-rays were designed mainly to assess delays or advancement in maturation of the bone, knowing the individual’s chronological age rather than as a means of testing chronological age. Skeletal age is indeed a person’s age measured by matching bone development (as shown by X-rays) with bone development of an average person of known chronological age.

During age-determination practices in forensic settings, physicians often estimate the bone and dental ages, and interpret them as the chronological ages that would have been attained if the individual had been maturing at the same rate as the individuals of identical sex and reference population (62). But the accuracy of the age estimation is greatly influenced by sexual and ethnic variability, and the necessity to use sex- and population-specific standards has been recently emphasized by Schmitt et al. (61), suggesting that national standards should be established and updated regularly if bone age or dental age are to be used to assess development.

The issue of which reference population to use in assessing the adequacy of growth during childhood is crucial in clinical settings as the appropriateness of the reference charts needs to be regularly reviewed. For example, the original Tanner and Whitehouse charts for height and weight (10) are actually obsolete because of the well-recognized secular trend toward increasing height at all ages (63). They have been replaced recently by more recent growth charts, such as those developed in the United States by the National Center for Health Statistics, with improved data and statistical procedures (64). British modern growth charts are also available (65), and boys and girls are usually plotted on different charts because their growth rates and patterns differ.

Even if methods elaborated in forensic sciences are most often based on sex- and population-specific standards representing individuals from the major geographical regions of the world, the variability between individuals within the same sex and population usually represents a common source of error in

age assessment, often underestimated (66). Variation in the biological aging process has profound effects on age assessment in living individuals as well as at death (61), and sometimes the margin of error in correlating biological age with chronological age is too great to permit accurate forensic conclusions. Remarkable differences (up to 3 yr) between bone age and chronological age have been observed in healthy subjects, probably reflecting the effect of the secular trend toward earlier maturation or alterations in pubertal development (62).

A discrepancy between the chronological age and the bone age can also indicate an atypical skeletal development. For example, Himes (67) demonstrated that chronic malnutrition can delay maturation by 2 or 3 yr. Even poor hygienic conditions can produce a retarding effect of skeletal maturation, demonstrating how environment or socioeconomic status can deeply affect the rate of general growth more than genetic or ethnical factors. In fact, it is well known that there is a genetically determined potential of skeletal maturation that does not depend on ethnicity and is available for exploitation under optimal environmental conditions (i.e., high socioeconomic status), whereas a less favorable environment may lead a significant delay in skeletal maturation (68). Like any other human biological characteristic, dental and bone formation has a regional or large-scale geographic variation, which is (sometimes inappropriately) called “ethnicity.”

Ethnic origin, in particular, seems to influence much more tooth mineralization rather than skeletal maturation, as demonstrated by a comparative study of wisdom tooth on three population samples (German, Japanese, and South African). The use of population-specific standards can especially enhance the accuracy of forensic age estimates based on wisdom tooth mineralization (69). On the other hand, a recent investigation on the effects of ethnicity on skeleton and related consequences for forensic age estimations concluded that skeletal maturation takes place in phases that are identically defined for all ethnic groups (68). Time-related differences in passing those stages of skeletal maturation within the relevant age group appear to be unaffected by ethnic identity, but the socioeconomic status of a given population seems to be of decisive importance to the rate of ossification. Thus, the application of X-ray standards to an individual of a socioeconomic status lower than that of the reference population can lead to underestimation of that person’s age. The risk of underestimation of age should be underlined in any expert opinion to protect the person.

3. COMBINED METHODS AND STATE OF THE ART

Because the level of reliability and accuracy in age assessment is highly variable between studies, it appears that no stable method exists. None of the

modern techniques are both easy to use and practical for forensic practitioner as most of them result in a slight over- or underestimation, depending on the applied method and discrepancies between biological and legal age. Even the most thorough medical tests cannot provide conclusive evidence of a young person's age as they measure maturity, not chronological age. Furthermore, no single maturity indicator of age is ever likely to reflect accurately the many factors that accumulate with chronological age, each of which can contribute valuable information to the age estimate in living as well in dead people (70). Therefore, the investigator should use all the available evidence, including multiple age indicators as multifactor techniques are recommended by many authors (71).

Ubelaker (30) sagely suggests that all appropriate techniques must be employed before reaching a decision, to minimize the effects of factors not directly related to the aging process. Special emphasis should be given to age indicators with the highest correlation with chronological age, and any final estimate should express the known variability in the aging process, especially with regard to the sex and population affinity of the individual (31). In this matter, Pfau and Sciulli (72) introduced a promising radiographic method for age estimation, using multiple criteria designed mainly for subadults and useful for the formation of age standards for a specific population.

Therefore, sexual changes, bones, and teeth can be actually considered (all together) the only reliable indices in assessing a living individual's age in forensic settings. Comprehensive approaches to age estimation that consider multiple maturity indicators are superior to isolated methods (73), and their success reflects not only the morphological expression of the aging process, but also the technique complexity and the experience of the investigator.

Regarding the combination of procedures for age assessment, a multimedia database is also available. It is the Electronic Encyclopedia on Maxillo-Facial, Dental and Skeletal Development developed by Demirjian (74), which contains almost 4000 digitalized X-rays collected between 1966 and 1981 at the Growth Center of the University of Montreal and divided in two sets: babies (from birth to age 6 yr) and adolescents (from 6 to 17 yr). The encyclopedia, in CD-ROM format, provides an archive of anthropometric and cephalometric data, and dental and skeletal X-rays, which is often of great value as a reference.

The most obvious application of the data contained in the CD-ROM is in comparing observations and measurements obtained from a set of unknown individuals to information obtained from children of known ages (74). The data can also be studied from a statistical point of view by using percentile values. The encyclopedia also includes programs for direct calculation of age,

separately for males and females. When searching by age, three different assignments are given to every child: chronological, skeletal, and dental. Skeletal age is calculated by the TW2-RUS methods, whereas dental age is based on the Demirjian's systems. The user can easily move between X-rays and measurements of subjects at a given chronological age, comparing dental and skeletal development to his or her unknown, or move through the dental or skeletal X-rays of a specific individual, comparing his or her unknown to individuals at various chronological ages. By using cross-sectional or longitudinal studies, everyone can appreciate the tremendous variation intrinsic to the human growth process.

According to Ritz-Timme et al. (75), it is surprising how few attempts have been made to find common standardization, calibration, and evaluation procedures for age estimation in living individuals as well in human remains, especially in the beginning of this century, in which quality control has achieved a great importance in all fields of the biomedical sciences. Actually, there are no generally accepted guidelines concerning quality assurance in age estimation. Efforts in this direction are necessary in order to guarantee adequate answers to the important legal and social issue of age estimation in forensic medicine.

In age estimation of living individuals, the only guidelines available are those developed by the international and interdisciplinary Study Group on Forensic Age Estimation (76), constituted in Berlin in March 2000, which currently has 76 members from Germany, Belgium, France, Norway, Spain, and the United States. This is the only official step made by a scientific community for issuing expert opinions in order to standardize the yet rather heterogeneous procedure and to implement quality assurance policies in this area (3). According to recommendations of the Study Group, every expert opinion must comprise three independent parts contributed by forensically experienced physicians of the relevant disciplines (auxology, radiology, and dentistry). These include the physical examination, the X-ray evaluation of the left hand by a radiologist, and a dental examination that records dentition status and evaluates an orthopantomogram. An additional X-ray examination or computed tomography scan of the clavicle is also recommended to establish whether a person has reached the age of 21, based on the fusion of the medial epiphysis.

The physical examination determines anthropometric measures, such as stature and weight, along with signs of sexual maturity and identification of any developmental disorders that might affect age-appropriate development; Tanner's sexual maturity rating (6,7) is commonly used for this purpose. The mineralization of the third molars is assessed using the information stages

described by Demirjian et al. (21), modified in accordance with Mincer et al. (17). Criteria for evaluating left-hand radiographs include the form and size of bone elements and the degree of ossification of epiphyseal cartilages. Radiographic atlas and/or single bone methods such as the G&P (36) and TW2 (41) techniques are used. The X-ray image is compared with standards of the relevant age and sex, thus, degree of skeletal maturity is determined for selected bones.

The accuracy of the combined method proposed by the Study Group has been evaluated by statistical analysis, concluding that deviation between estimated and true age ranged between ± 12 mo in most of the cases (77). These results confirm how difficult is to determine exactly the age of living individual, even based on a combination of techniques using multiple maturity indicators. In this respect, the Study Group has emphasized that the central forensic aspect of an expert report is giving the most probable age of the individual examined and/or the degree of probability that the stated age is the actual age or that the individual's age is higher than the relevant penal age limit. Reference studies on which the age estimation is based have to be necessarily quoted in the final report.

Because in forensic science mere visual estimates are simply unreliable and unacceptable, the use of validated and scientifically based formal methods is crucial for every forensic evaluation (78). The age-assessment procedure developed by the Study Group can be considered a valuable approach to give some objectivity to age-determination practices, even if some remarks can be highlighted. They concern mainly the choice of standards for sexual maturation and for hand and wrist X-rays. For example, Tanner growth charts for height and weight (10), as well the G&P atlas (36), are no longer reliable for use and could be inaccurate because they do not represent a modern multi-ethnic pediatric population. Furthermore, different population-specific standards should be considered or, even better, new reference studies should be developed. With respect to the quality control in forensic age-determination practice, more studies are needed to test the reliability of existing techniques on samples other than those originally used to develop the technique or formulae.

Another statement deals with the radiographic analysis of teeth and bones and therefore the applicability of additional X-rays (such as those recommended for the clavicle): radiography plays a very important role for the inspection of dental maturity and skeletal development and, indeed, it is of invaluable assistance in the age-estimation practice. Nevertheless, a radiograph is, after all, a diagnostic intrusive procedure and must be guided by principles of medical ethics.

Dental X-rays or hand and wrist X-rays involve always a minimal radiation exposure, and precautions must be necessarily taken when radiographs are used, in particular, for pregnant girls. An alternative procedure of skeletal age assessment has been proposed using a less invasive method such as dual-energy X-ray absorptiometry, and a high degree of agreement of bone age assessments based on dual-energy X-ray absorptiometry and radiographic images has been observed (79).

However, regarding the radiographic analysis in the assessment of age, since 1996 the Royal College of Radiologists (80) gave useful advice to its members, arguing strongly that ionizing radiation should be used only in “cases of clinical need.” In fact, X-rays should be considered unjustified if requested only to ascertain the alleged chronological age. Otherwise, X-ray examination that is not based on medical indication has to be authorized by an authority order.

With respect to the principles of patient autonomy, welfare, and consent, some other invasive procedures, such as dentine sampling by tooth extraction or biopsy in living individuals, even if strongly recommended based on their high accuracy (75,81), should be also considered unjustified especially if forced against the individual’s wishes. It is true that methods for sectioned teeth seem to give more reliable results when compared with methods for intact teeth (78), but this practice can only be considered depending on the circumstances of individual cases (i.e., loss of single tooth), and in adulthood, when the criteria for the aging process, such as the eruption and the development of roots, are not available. In fact, once the adult dentition is fully formed, accurate age assessment by dental means becomes difficult without the removal of a tooth for microscopic analysis.

In order to increase the accuracy of age estimates and to identify age-relevant development disorders, a combination of methods, such as those mentioned previously, is strongly recommended. However, even using a multifactor technique, the authors believe that the margin of error seems too great to permit reasonable conclusions in forensic settings. The poor correlation between maturity and chronological age represents a fundamental limitation to age-determination practices, especially for forensic purposes. Based on such inaccurate estimates, authorities could incarcerate some children in adult detention centers, which are unsafe and inappropriate for minors.

In this respect, in a letter to the Department of Homeland Security, several American leaders in dentistry, medicine, and psychology have recently expressed their strong concern over irresponsible age-determination practices affecting the lives of young immigrants, including asylum seekers (82). In a public comment to American Immigration and Naturalization Service, Ferraro

(58) also expressed the danger of the current age-determination practice in forensic settings, because of its apparent accuracy that is given an unwarranted scientific legitimacy (58).

4. CONCLUSION

According to Maples (83), age determination is ultimately an art, not a precise science. The margin of error can be substantial in living individuals, sometimes by as much as 2–3 yr either side, or at best, 12 mo, even by using combined methods (77). On the other hand, estimates of age may lose credibility if they are too precise, because many biological variables in humans exist to achieve reliable results, and there are several hazards in any age-estimation method. Even the most thorough medical tests cannot provide conclusive evidence of a young person's age as they measure maturity, not chronological age. Therefore, no age-determination technique would ever be extremely precise and accurate because human development is complex and variable.

In the Guidelines for Paediatricians (84), the Royal College of Paediatrics already focused on how age determination is extremely difficult to do with certainty, and no single approach to this can be relied on. There may be difficulties in determining whether a young person who might be as old as 23 yr could, in fact, be under the age of 18. For young people aged 15–18 yr, it is even less possible to be certain about age.

As there can be a wide margin of error in assessing age; it may be best to word a clinical or forensic judgment in terms of whether a child is probably, likely, possibly or unlikely to be under the age of 14 or 18. According to the Guidelines for Paediatricians (84) forms of sentences such as “Her/his age may be in the range x – y years,” or “He/she is likely to be the age that he/she claims for the following reasons: [give reasons],” may be appropriate. According to recommendations of the Study Group on Forensic Age Estimation, the estimates should necessarily refer to the margin of error or the possible range of variation around the mean associated with that particular method adopted, and reference studies on which the age estimation is based have to be necessarily quoted in the final report. Nevertheless, when the exact age is uncertain, the child should be given the benefit of the doubt.

In conclusion, every investigator must approach each case with caution regarding the discrepancies between biological and legal age, the range of human variation between sex and population, the intervention of pathological conditions, and the appropriate application of standards discussed and presented in this chapter.

REFERENCES

1. Schmeling, A., Olze, A., Reisinger, W., Geserick, G. Age estimation of living people undergoing criminal proceedings. *Lancet* 358:89–90, 2001.
2. United Nations High Commissioner for Refugees. Guidelines on policies and procedures in dealing with unaccompanied children seeking asylum. United Nations High Commissioner for Refugees, Geneva, February 1997.
3. Schmeling, A., Olze, A., Reisinger, W., Geserick, G. Forensic age estimation of living people undergoing criminal proceedings. *Forensic Sci. Int.* 144:243–245, 2004.
4. Vaughan III, C., Litt, I. F. Developmental pediatrics: assessment of growth and development. In: Behrman, R. E., Vaughan III, C., eds., *Nelson Textbook of Pediatrics*, 13th Ed., WB Saunders, Philadelphia, PA, pp. 24–33, 1987.
5. Pietrobelli, A., Faith, M. S., Allison, D. B., Gallagher, D., Chiumello, G., Heymsfield, S. B. Body mass index as a measure of adiposity among children and adolescents: a validation study. *J. Pediatr.* 132:204–210, 1998.
6. Marshall, W. A., Tanner, J. M. Variations in pattern of pubertal changes in girls. *Arch. Dis. Child.* 44:291–303, 1969.
7. Marshall, W. A., Tanner, J. M. Variations in pattern of pubertal changes in boys. *Arch. Dis. Child.* 45:13–23, 1970.
8. Feik, S. A., Glover, J. E. Growth of children's faces. In: Clement, J. G., Ranson, D. L., Ranson, D., eds., *Craniofacial Identification in Forensic Medicine*. Arnold, London, pp. 203–224, 1998.
9. Iscan, M. Y., Kennedy, K. A. R. Reconstruction of life from the skeleton. Liss, New York, pp. 109–127; 191–200, 201–222, 1989.
10. Tanner, J. M., Whitehouse, R. H. Clinical longitudinal standards for height, weight, height velocity and weight velocity and stages of puberty. *Arch. Dis. Child.* 51: 170–179, 1976.
11. Hammer, L. D., Kraemer, H. C., Wilson, D. M., Ritter, P. L., Dornbusch, S. M. Standardized percentile curves of body-mass index for children and adolescents. *Am. J. Dis. Child.* 145:259–263, 1991.
12. Valadian, I., Porter D. *Physical Growth and Development from Conception to Maturity*. Little Brown, Boston, MA, 1977.
13. Wright, C. M., Booth, I. W., Buckler, J. M. H., et al. Growth reference charts for use in the United Kingdom. *Arch. Dis. Child.* 86:11–14, 2002.
14. Krogman, W. M., Iscan, M. Y. *The Human Skeleton in Forensic Medicine*. Charles C. Thomas Publisher, Springfield, IL, pp. 50–102, 1986.
15. Hagg, U., Matsson, L. Dental maturity as an indicator of chronologic age. The accuracy and precision of three methods. *Eur. J. Orthod.* 7:25–34, 1985.
16. Mincer, H. H., Harris, E. F., Berryman, H. E. Molar development as an estimator of chronologic age. In: Bowers, C. M., Bell, G. L., eds., *Manual of Forensic Odontology*, 3rd Ed. American Society of Forensic Odontology, Saratoga Springs, NY, 86–89, 1997.
17. Mincer, H. H., Harris, E. F., Berryman, H. E. The ABFO study of third molar development and the use as an estimator of chronological age. *J. Forensic Sci.* 38:379–390, 1993.

18. Kullman, L., Johanson, G., Akesson, L. Root development of the lower third molar and its relation to chronological age. *Swed. Dent. J.* 16:161–167, 1992.
19. Levesque, G.-Y., Demirjian, A., Tanguay, R. Sexual dimorphism in the development, emergence and agenesis of the mandibular third molar. *J. Dent. Res.* 60:1735–1741, 1981.
20. Gleiser, I., Hunt, Jr., E. E. The permanent mandibular first molar: its calcification, eruption and decay. *Am. J. Phys. Anth.* 13:253–281, 1955.
21. Demirjian, A., Goldstein, H., Tanner, J. M. A new system of dental age assessment. *Hum. Biol.* 45:211–227, 1973.
22. Mesotten, K., Gunst, K., Carbonez, A., Willems, G. Dental age estimation and third molars: a preliminary study. *Forensic Sci. Int.* 129:110–115, 2002.
23. Solari, A., Abramovitch, K. The accuracy and precision of third molar development as an indicator of chronological age in Hispanics. *J. Forensic Sci.* 47:531–535, 2002.
24. Gunst, K., Mesotten, K., Carbonez, A., Willems, G. Third molar root development in relation to chronological age: a large sample sized retrospective study. *Forensic Sci. Int.* 136:52–57, 2003.
25. Foti, B., Layls, L., Adalian, P., et al. New forensic approach to age determination in children based on tooth eruption. *Forensic Sci. Int.* 132:49–56, 2003.
26. Thorson, J., Hagg, U. The accuracy and precision of the mandibular molar as an indicator of chronological age. *Swed. Dent. J.* 15:15–22, 1991.
27. Kullman, L. Accuracy of two dental and one skeletal age estimation method in Swedish adolescents. *Forensic Sci. Int.* 75:225–236, 1995.
28. Tanner, J. M. Physical growth and development. In: Forfar, J. O., Arnell, C. C. eds., *Textbook of Pediatrics*, 2nd Ed. Churchill Livingstone, Edinburgh, pp. 249–303, 1978.
29. Liversidge, H. M., Herdeg, B., Rosing, F. W. Dental age estimation of non-adults: a review of methods and principals. In: Alt, K. W., Rosing, F. W., Teschler-Nicola, M., eds., *Dental Anthropology. Fundamentals, Limits and Prospects*. Springer, Vienna, pp. 420–422, 1998.
30. Ubelaker, D. H. The estimation of age at death from immature human bone. In: Iscan, M. Y. Ed., *Age Markers in the Human Skeleton*. Charles C. Thomas, Springfield, IL, pp. 55–70, 1989.
31. Lovejoy, C. O., Meindl, R. S., Mensforth, R. P., Barton, T. J. Multifactorial determination of skeletal age at death: a method and blind tests of its accuracy. *Am. J. Phys. Anthropol.*, 68:1–14, 1985.
32. Rösing, F. W., Kvaal, S. I. Dental age in adults: a review of estimation methods. In: Alt, K. W., Rosing, F. W., Teschler-Nicola, M. eds., *Dental Anthropology. Fundamentals, Limits and Prospects*. Springer, Vienna, pp. 443–468, 1998.
33. Helm, S. Relationship between dental and skeletal maturation in Danish schoolchildren. *Scand. J. Dent. Res.* 98:313–317, 1990.
34. Cox, L. A. The biology of bone maturation and ageing. *Acta Paediatr.* 423:S107–S108, 1997.
35. Zerlin, J. M., Hernandez RJ. Approach to skeletal maturation. *Hand Clin.* 7:53–62, 1991.

36. Greulich, W. W., Pyle, S. I. Radiographic Atlas of Skeletal Development of the Hand and Wrist, 2nd Ed. Stanford University Press, Stanford, CA, pp. 9–13, 1959.
37. Ubelaker, D. H. Estimating age at death from immature human skeletons: an overview. *J. Forensic Sci.* 32:1254–1263, 1987.
38. Loder, R. T., Estle, D. T., Morrison, K., et al. Applicability of the Greulich and Pyle skeletal age standards to black and white children of today. *Am. J. Dis. Child.* 147:1329–1333, 1993.
39. Ontell, F. K., Ivanovic, M., Ablin, D. S., Barlow, T. W. Bone age in children of diverse ethnicity. *Am. J. Roentgenol.* 167:1395–1398, 1996.
40. Mora, S., Boechat, M. I., Pietka, E., Huang, H. K., Gilsanz, V. Skeletal age determinations in children of European and African descent: applicability of the Greulich and Pyle standards. *Pediatr. Res.* 50:624–628, 2001.
41. Tanner, J. M., Whitehouse, R. H., Marshall, W. A., Healy, M. J. R., Goldstein, H. Assessment of skeletal maturity and prediction of adult height (TW2 method). Academic, London, 1975.
42. Acheson, R. M. A method of assessing skeletal maturity from radiographs. A report from Oxford Child Health Survey. *J. Anat.* 88:498–515, 1954.
43. Roche, A. F., Johnson, J. M. A comparison between methods of calculating skeletal age (Greulich-Pyle). *Am. J. Phys. Anthropol.* 30:221–230, 1969.
44. Roche, A. F., Davila, G. H. The reliability of assessments of the maturity of individual hand-wrist bones. *Hum. Biol.* 48:585–590, 1976.
45. Bull, R. K., Edwards, P. D., Kemp, P. M., Fry, S., Hughes, I. A. Bone age assessment: a large scale comparison of the Greulich and Pyle, and Tanner and Whitehouse (TW2) methods. *Arch. Dis. Child.* 81:172–173, 1999.
46. King, D. G., Stevenson, D. M., O'Sullivan, M. P., et al. Reproducibility of bone ages when performed by radiology registrars: an audit of Tanner and Whitehouse II versus Greulich and Pyle methods. *Br. J. Radiol.* 67:848–851, 1994.
47. Cox, L. A. Tanner-Whitehouse method of assessing skeletal maturity: problems and common errors. *Horm. Res.* 45:53–55, 1996.
48. Sorg, M. H., Andrews, R. P., Iscan, M. Y. Radiographic aging of the adult. In: Iscan, M. Y., Ed., *Age Markers in the Human Skeleton*. Charles C. Thomas, Springfield, IL, pp. 169–193, 1989.
49. Drennen, M. R., Keen, J. A. Identity. In: Gordon, I., Turner, R., Price, T. W., eds., *Medical Jurisprudence*. Livingston, Edinburgh, pp. 336–372, 1953.
50. Krogman, W. M., Iscan, M. Y. The human skeleton in forensic medicine. Charles C. Thomas Publisher, Springfield, IL, 458–479, 1986.
51. Tanner, J. M., Gibbons, R. D. Automatic bone age measurement using computerized image analysis. *J. Pediatr. Endocrinol.* 7:141–145, 1994.
52. Tanner, J. M., Gibbons, R. D. A computerized image analysis system for estimating Tanner-Whitehouse 2 bone age. *Horm. Res.* 42:282–287, 1994.
53. Frisch, H., Riedl, S., Waldhor, T. Computer-aided estimation of skeletal age and comparison with bone age evaluations by the method of Greulich-Pyle and Tanner-Whitehouse. *Pediatr. Radiol.* 26:226–231, 1996.
54. Niemeijer, M., van Ginneken, B., Maas, C., Beek, F. J. A., Viergever, M. A. Assessing the skeletal age from a hand radiograph: automating the Tanner-Whitehouse

- method. In: Sonka, M., Fitzpatrick, J. M. eds., *Medical Imaging 2003: Image Processing*. SPIE, pp. 1197–1205, 2003.
55. Roche, A. F., Chumlea, W. C., Thissen, D. Assessing the skeletal maturity of the hand-wrist: FELS method. Springfield, IL, Charles C. Thomas Publisher, 1988.
56. Gilli, G. The assessment of skeletal maturation. *Horm. Res.* 45:45–52, 1996.
57. Tanner, J. M., Healy, M. J. R., Goldstein, H., Cameron, N. Assessment of skeletal maturity and prediction of adult height (TW3 method). Saunders, 2001.
58. Ferraro, N. F. Comment to the Immigration and Naturalization Service (INS) regarding determination of chronological age using bone age and dental age standards. Public comment on proposed Rule at 64 FR 39759 (INS No. 1906-98) In: *From Persecution to Prison: The Health Consequences of Detention for Asylum Seekers*. Physicians for Human Rights (PHR), June 2003. Also available on the PHR website at http://www.phrusa.org/campaigns/asylum_network/detention_execSummary/detention_pdf.pdf. Last accessed on Feb. 15, 2006.
59. Van Lenthe, F. J., Kemper, H. C., van Mechelen, W. Skeletal maturation in adolescence: a comparison between the Tanner-Whitehouse II and the Fels method. *Eur. J. Pediatr.* 157:798–801, 1998.
60. Baer, M. J. Growth and maturation. An introduction to physical development. Doyle, Cambridge, MA, 1977.
61. Schmitt, A., Murail, P., Cunha, E., Rougé, D. Variability of the pattern of aging on the human skeleton: evidence from bone indicators and implications on age at death estimation. *J. Forensic Sci.* 47:1203–1209, 2002.
62. Johnston, F. E., Zimmer, L. O. Assessment of growth and age in the immature skeleton. In: Iscan, M. Y., Kennedy, K. A. R., eds., *Reconstruction of Life from the Skeleton*. Liss, New York, NY, pp. 11–40, 1989.
63. Rona, R., Chinn, S. The national study of health and growth. Oxford University Press, Oxford, 1999.
64. Kuczmarski, R. J., Ogden, C. L., Guo, S. S., et al. CDC growth charts for the United States: methods and development. *Vital Health Stat.* 11:1–190, 2002.
65. Cameron, N. British growth charts for height and weight with recommendations concerning their use in auxological assessment. *Ann. Hum. Biol.* 29:1–10, 2002.
66. Ubelaker, D. H. Methodological consideration in the forensic applications of human skeletal biology. In: Katzenberg, M. A., Saunders, S. R. eds., *Biological Anthropology of the Human Skeleton*. Wiley-Liss, New York, NY, pp. 41–67, 2000.
67. Himes, J. H. Bone growth and development in protein-calorie malnutrition. *World Rev. Nutr. Diet.* 28:143–187, 1978.
68. Schmeling, A., Reisinger, W., Loreck, D., Vendura, K., Markus, W., Geserick, G. Effects of ethnicity on skeletal maturation: consequences for forensic age estimations. *Int. J. Legal Med.* 113:253–58, 2000.
69. Olze, A., Schmeling, A., Taniguchi, M., et al. Forensic age estimation in living subjects: the ethnic factor in wisdom tooth mineralization. *Int. J. Legal Med.* 118:170–173, 2004.
70. Meindl, R. S., Lovejoy, C. O. Ectocranial suture closure: a revised method for the determination of skeletal age at death based on the lateral-anterior sutures. *Am. J. Phys. Anthropol.* 68:57–66, 1985.

71. Bedford, M. E., Russel, K. F., Lovejoy, C. O., et al. Test of the multifactorial aging method using skeleton with known age-at-death from the Grant collection. *Am. J. Phys. Anthropol.* 91:287–297, 1993.
72. Pfau, R. O., Sculli, P. W. A method for establishing the age of subadults. *J. Forensic Sci.* 39:165–176, 1994.
73. Baccino, E., Ubelaker, D. H., Hayek, L.-A., Zerilli, A. Evaluation of seven methods of estimating age at death from mature human skeletal remains. *J. Forensic Sci.* 44:931–936, 1999.
74. Reichs, K. J., Demirjian, A. A multimedia tool for the assessment of age in immature remains: the electronic encyclopedia for maxillo-facial, dental and skeletal development. In: Reichs, K. J., Ed., *Forensic Osteology. Advances in the Identification of Human Remains*. Charles C. Thomas Publisher, Springfield, IL, pp. 253–267, 1998.
75. Ritz-Timme, S., Cattaneo, C., Collins, M. J., et al. Age estimation: the state of the art in relation to the specific demands of forensic practise. *Int. J. Legal Med.* 113: 129–136, 2000.
76. <http://www.charite.de/rechtsmedizin/agfad/empfehlungen1.html#Englisch>. Last accessed on Feb. 15, 2006.
77. Schmeling, A., Olze, A., Reisinger, W., König, M., Geserick, G. Statistical analysis and verification of forensic age estimation of living persons in the Institute of Legal Medicine of the Berlin University Hospital Charité. *Legal Med.* 5:S367–S371, 2003.
78. Soomer, H., Ranta, H., Lincoln, M. J., Penttilä, A., Leibur, E. Reliability and validity of eight dental age. Estimation methods for adults. *J. Forensic Sci.* 48:149–152, 2003.
79. Pludowski, P., Lebedowski, M., Lorenc, R. S. Evaluation of the possibility to assess bone age on the basis of DXA derived hand scans—preliminary results. *Osteoporos Int.* 15:317–322, 2004.
80. Watt, I., Faculty of Clinical radiology. Letter ref. BFCR 9 to all Home Clinical radiology Fellows and Members, 1996.
81. Ritz, S., Stock, R., Schutz, H. W., Kaatsch, H.-J. Age estimation in biopsy specimens of dentin. *Int. J. Legal Med.* 108:135–139, 1995.
82. Letter from American Leaders in Dentistry, Medicine, and Psychology Expressing Concern Over Irresponsible Age Determination Practices Affecting the Lives of Young Immigrants, Including Asylum Seekers to the Department of Homeland Security. Available on the Physicians for Human Rights website at http://www.phrusa.org/campaigns/asylum_network/pdf/age-testingletter.pdf. Last accessed on Feb. 15, 2006.
83. Maples, W. R. The practical application of age-estimation techniques. In: Iscan, M. Y., Ed., *Age Markers in the Human Skeleton*. Charles C. Thomas Publishers, Springfield, IL, pp. 319–324, 1989.
84. Levenson, R., Sharma, A. The health of refugee children. Guidelines for Paediatricians. Royal College of Paediatrics and Child Health, London, 1999.