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# Forensic Age Estimation in Unaccompanied Minors and Young Living Adults

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## 1. Introduction

### 1.1 FAE a need in nowadays everyday forensic practice

Forensic Age Estimation (FAE) defines an expertise in forensic medicine which aims to define in the most accurate way the chronological age of a person of an unknown age involved in judicial or legal proceedings. The term “estimation” defines more precisely than other as “diagnosis” the real limits inherent to this expertise. The state of the art in FAE is such that nowadays there is no medical test or a group of tests that absolutely and accurately let us know the exact chronological age of a human being (Ritz Timme et al., 2000).

Nevertheless, in everyday practice, Justice Courts and other Public Institutions require this type of experts reports to forensic physicians and Legal Medicine Institutes. In this expertise it's needed a collaborative knowledge of very diverse disciplines like Forensic and Physical Anthropology, Odontology and even some general medical specialities like Radiology of Pediatrics all of them ideally centered in Legal Medicine Institutes.

FAE is not at all a recent field of expertise in Forensic Sciences and Judiciary History. In old Roman Empire, the eruption of second molar indicated the moment in which a young male was considered for military service (Schmeling, 2008). During XIX century age estimation was mainly performed by dentists. In 1837, Edwing Saunders published “The Teeth a Test of Age” and British Parliament decided to teeth eruption as an accurate method to determine the age of kids. At those ages, minimum criminal age was 7 years old in Britain and also in 1883 minimum required age for mining workers was 9 years. Nevertheless, at that time, also some voices expressed their criticism with this practice for an age estimation. In 1846, Dr. Pedro Mata in his text book of Legal Medicine expressed his concern on assuring without any kind of a doubt an age estimation based solely on teeth eruption (Mata, 1846).

In 1895, Röntgen discovered X rays. His discover opens a new dimension for age estimation in living subjects. The applications of his discovery in Legal Medicine were nearly

immediate and age estimation in living subjects rapidly changed to include these new tests based on radiographies of the skeleton as a complement to the teeth eruption classical methods. In 1886 in Munchen, Angerer was first to suggest that radiographies from carpus could be indicator of an age in young persons (Brogdon, 1998). In 1887, Behrendsen published the first systematic review of age variations in carpus (Schmeling, 2008).

During the next 40 years, different researches focused in defining the standard radiological maturation of human skeleton with age (Stevenson, 1924; Flecker, 1933; Galstaun, 1937; Sidhom & Derry, 1931; Pryor, 1908; Borovansky & Hněvkovsky, 1927; Davies & Parsons, 1927; Paterson, 1929; Meenes & Holly, 1932; Adair & Scammon, 1921). The lack of consciousness on the harms inherent to X-rays exposure lead to a massive amount of X-rays expositions only on investigation purposes. Between decades of 50 to 80 of XX century, main definitive methods of age estimation based on radiological analysis of carpus (Greulich & Pyle, 1959; Tanner et al, 1983) and dental maturation (Nolla, 1960; Demirjian, 1975) were defined.

Until recent years, forensic physicians in European Countries weren't usually required for a FAE in living subjects. Census and Birth Registration in Europe was really accurate and only exceptionally an expert report was needed to assess an age of a European citizen. But in the last two decades European countries have received a flood of immigrants from extracommunitarian countries. In many cases these non European citizens don't have any documents that ascertain their chronological age or there are doubts on the certainty of chronological age alleged. This situation is specially complex in a case of an immigrant supposedly minor of age. European Legislation and International Traits assure an special treatment of these immigrant minors when they are unaccompanied and they must be consider under the trusteeship of European authorities. This special treatment must also be assured in case of criminal proceedings in which minors have a special protection as victims and also as responsible of criminal activities. In all those cases, Justice Courts and Public Institutions require forensic doctors a FAE expert report (European Migration Network, 2010).

It's very difficult to ascertain the total number of immigrants supposedly minors unaccompanied with an known chronological age in European Countries. In our legal framework, unaccompanied minors is the term to define this population. There are many different reasons in each European country to determine the uncertainty of a statistics on the total number of unaccompanied minors. In countries like Germany or Spain, the division of the Government in Lander or Autonomous Communities turn difficult to maintain a central register of minors unaccompanied. In most European Countries it was also observed that many minors under trusteeship of authorities suddenly disappear from the asylum institutions. Some studies also stress the fact that there is a group of minors unaccompanied who don't seek for asylum and they keep as immigrants in many European cities unknown to the authorities (European Migration Network, 2010).

During 2008 in Germany the census of minors unaccompanied was a total of 763 subjects. From these 324 were up to 15 years old and 438 were aged 16 to 17. Most of them were detected at the airports and they came mainly from Iraq and Afghanistan during 2007 and 2008. During 2003 to 2006, the most usual nationalities were Turkish and Chinese, and other nationalities more unusual were Russian, Serbian, Vietnamese and Nigerian (Federal Office for Migration and Refugees, 2009).

In Spain, the census of unaccompanied minors by the end of 2008 was an estimation of 6000 minors under trusteeship of Spanish authorities. Nearly all of them came from Magreb and

Sahel African Subsaharian countries, being most usual nationality Moroccan. Until 2003 most of them arrived at Spain inside trucks and industrial vehicles crossing Mediterranean Sea in Algeciras (Andalucia). Since 2003, this changed and most of the minors detected arrived at Southern Spanish and Canary Islands coast by sea in “pateras” or “cayucos”, different light and dangerous boats that illegally cross Atlantic and Mediterranean sea waters (Bravo, 2005; PICUM, 2008).

In both cases, Spain and Germany, nearly all of the unaccompanied minors are male. Only in Somalian nationality girls are more frequent than boys (Bueno & Mestre, 2006; European Migration Network, 2010).

Nowadays, there is still no consensus in European countries on which methods must be applied when an age estimation of a supposedly minor is needed. All Member States attempt to determine this using a variety of techniques. European Statistical data are also uncertain about the methods really applied in every country. Nevertheless, official data from EU indicate that in some countries like United Kingdom authorities usually only apply an interview with the minor by a social worker without a medicine doctor examination. UK Border Agency accepts the “Merton compliant age assessment” carried out by two specially trained social workers when assessing the individual’s asylum claim, unless there is evidence to the contrary. On the other side, Austria since 2010 applies the so called “multifactorial examination methodology” that consists of three elements: an inspection by a doctor, a dental analysis and X-ray examinations, the latter performed only with the consent of the minor. If the age of the minor cannot be determined exactly, the benefit of the doubt is given to the individual concerned. In France, also a psychological interview is used for age estimations of unaccompanied minors. In six out of all Member States no skeletal development estimation by radiographies was applied and in other eight ones no dental analysis was performed (European Migration Network, 2010).

ACNUR has recently recommended EU authorities to unify methodology applied in age estimation to ensure the protection of children and the defense of immigrants Human Rights in Member States. Different international groups of experts have published guidelines and recommendations on FAE, like American Board of Forensic Odontologists (ABFO), International Organization for Forensic Odonto-Stomatology (IOFOS) or Study Group of Age Estimation of the German Society of Legal Medicine (AGFAD). This chapter aims to spread these recommendations on FAE by these international groups of experts (UNHCR, 2000,2001,2002).

## 2. Methods

### 2.1 Selection of the methods

In the infant and juvenile stage the use of morphological methods based on radiological examination of dental and skeletal development is recommended (Ritz Timme et al., 2000). However, the methodology employed varies, while the criteria followed internationally for official application of these techniques are disparate, with some countries arguing the dubious validity that much scientific research currently accords this type of proof, whose margins of error do not permit the diagnostic reliability required in such cases (UNHCR, 2000, 2001, 2002). For this reason, in certain countries X-ray tests are only used in criminal cases (Solari & Abramovitz, 20202). Despite this, the programs developed by official institutions and NGOs have established protocols of good practice which include the elements corresponding to age assessment (UNHCR, 1997).

In accordance to the guidelines proposed by Ritz-Timme et al for an age assessment method to be considered acceptable, it must fulfill the following requirements:

1. The method must be transparent and provable, presented to the scientific community, as a rule by publication in peer-reviewed journals.
2. Clear information concerning accuracy of age assessment by the method should be available.
3. The method needs to be sufficiently accurate to fulfill the specific demands of the single case to solve the underlying questions.
4. In cases of age assessment in living individuals principles of medical ethics and legal regulations have to be considered, especially if medical intervention is involved.

Reference material used must fulfill certain requirements (Solari & Abramovitch, 2002):

- Adequate sample size. The number of subjects of each sex and age group should be ten times the number of the examined features.
- The age indicated by the subjects should be verified.
- An even distribution of subjects across all age groups.
- All data have to be collected separately for both sexes.
- The time of the examination should be recorded.
- The examined features should be defined unambiguously.
- The technique used in the examination should be described precisely.
- Information on genetic-geographic origin, socio-economic status and health of the reference population is indispensable.
- The sample size, mean value and statistical parameters of deviation should be provided for every feature examined.
- Information on inter- and intra-observer error is desirable.

Meinl et al stand out in questioning the terms “genetic-geographic origin” “socio-economic status” and “state of health”, given the difficulty of establishing a definition of these parameters and the ethical aspects of these terms, which would condition their use in a reliable manner in a study (Meinl et al. 2007).

Many papers have been published concerning age estimation, and numerous different methods developed, although some of them have been considered inaccurate. The choice of a particular method will depend on the specific conditions of each case and mainly on the accuracy required.

In the opinion of the mentioned authors (Ritz-Timme et al. 2000), the published data which fulfill the demands listed above in childhood and adolescence, are those based on the examination of dental and/or skeletal development, applied by trained personnel. In childhood (0-14 years) radiological examination of dental development includes all tooth types of teeth. In adolescence (14-21 years), thirds molars are the only teeth undergoing maturation, resulting to a lesser degree in accuracy. In both cases, sex and race influence tooth development, so those factors have to be into account.

The German age study group considers that age diagnosis examination should include (AGFAD, 2001):

- A physical examination which also records anthropometric data, signs of sexual maturation and any age-relevant developmental disorder.
- An X-ray examination of the left hand.
- A dental examination which records dentition status and evaluates an orthopantomogram.



It also recommends that these methods are used in combination for the purpose of increasing accuracy in age assessment and to facilitate the identification of age-relevant developmental disorders (Schmeling et al. 2004).

Each part of the examination is recommended to be performed by a specialist experienced in setting up expert reports and participating in regular ring experiments (see below) for quality assurance, with a coordinating expert giving a comprehensive assessment on the basis of the different parts of the evaluation performed by the respective specialists (Garamendi et al. 2011).

### 3. Physical examination

Physical examination in cases of age determination should include measurements such as body height and weight, body type and body mass index, as well as any visible signs of sexual maturity and the results of a general physical examination, and should describe any signs suggestive of a pathological condition which may interfere with the maturation rate of the child.

There seems to be general agreement among authors that the interpretation of results obtained from anthropometric variables is an imprecise factor for the prediction of chronological age. Some studies have shown that individuals of greater height and weight and those with an athletic body type and an above-average BMI are among those who, in a specific population, may exhibit a more advanced bone age in relation to actual chronological age (Bueno et al. 1996).

Signs of sexual maturation are examined by evaluating the stage of development of the penis and scrotum, pubic hair growth, axillary hair growth, facial hair growth, and laryngeal prominence in male subjects; and breast development, axillary hair growth, and shape of the hip in female subjects.

The most widely used method for the study of secondary sexual characteristics is the staging described by Tanner (Marshall & Tanner, 1969, 1970). The method was devised to estimate the stage of development or physiological age for medical, educational or sports purposes, and to identify delayed or advanced sexual maturation when the chronological age of the subject is known. The method uses a five-stage scale to evaluate the status of pubic hair growth and breast development in girls, and pubic hair growth and development of the penis, scrotum and testes in boys.

Breast development stages (girls) (Marshall & Tanner, 1969):

- Stage 1: Prepubertal, papilla elevation only.
- Stage 2: Breast bud stage, elevation of breast and papilla as a small mound, enlargement of areola diameter.
- Stage 3: General enlargement of breast and areola.
- Stage 4: Projection of areola and papilla as secondary mound.
- Stage 5: Mature stage, adult contour with areola in same contour as breast and only papilla projecting.

Pubic hair growth stages (girls) (Marshall & Tanner, 1969) :

- Stage 1: Prepubertal, no pubic hair.
- Stage 2: Sparse growth of long, slightly pigmented, downy hair, straight or only slightly curled, chiefly along the labia.
- Stage 3: Considerably darker, coarser and more curled, with an increase in amount. The hair spreads sparsely over the junction of the pubes.

- Stage 4: Hair resembles adult type, but no spread to the medial surface of the thighs.
- Stage 5: Adult in quantity and type, spread to medial thighs.

Genital development stages (boys) (Marshall & Tanner, 1970):

- Stage 1: Prepubertal, no change in size or proportion of testes, scrotum and penis from early childhood.
- Stage 2: Enlargement of scrotum and testes, reddening and change in the texture of the scrotal skin.
- Stage 3: Increase first in length then breadth of penis, further growth of testes and scrotum.
- Stage 4: Enlargement in length and breadth of penis and development of glans, further growth of testes and scrotum, darkening of the scrotal skin.
- Stage 5: Genitalia adult in size and shape.

Pubic hair stages (boys) (Marshall & Tanner, 1970):

- Stage 1: Prepubertal, no pubic hair.
- Stage 2: Sparse growth of long, slightly pigmented, downy hair, straight or slightly curled, chiefly at the base of the penis.
- Stage 3: Considerably darker, coarser and more curled, with an increase in amount. The hair spreads sparsely over the junction of the pubes.
- Stage 4: Hair resembles adult type, but no spread to the medial surface of the thighs.
- Stage 5: Adult in quantity and type, spread to medial thighs.

Axillary hair growth, facial hair growth and laryngeal prominence development may also be assessed using the four-stage classification proposed by Nezy et al (Nezy et al, 1975).

Of the forensic methods recommended for age determination, assessing age on the basis of physical traits is the least precise. Evaluating sexual maturity has the greatest margin of error and should be used for age determination only in conjunction with an evaluation of skeletal maturity and tooth development. Multiple pathological conditions and non-pathological, idiosyncratic conditions cause a large range of variation in the onset of external changes associated with sexual maturation in different subjects. Therefore, age determination cannot be made on the basis of these examination data alone.

Moreover, irrespective of the difficulty in interpreting the results due to interobserver and intraobserver differences, there are few series analysing the progression of these parameters with chronological age in different populations, and the few available are mainly focused on developed countries (Koc et al, 2001; Cameron, 1993).

However, the physical examination is extremely useful for evaluating the potential impact of pathological factors on the maturation status estimated using other methods. The great discrepancy between height, weight and external signs of maturation and the bone and dental age estimated using radiographic methods should guide the examiner on the potential interference of pathological conditions and to a weighted estimation of age.

Most diseases delay development and are thus conducive to underestimation of age. Such underestimation of age would not disadvantage the person concerned in the judicial framework. By contrast, overestimating age due to a disease that accelerates development should be avoided at all costs. Certain diseases which occur very rarely, in particular endocrine disorders, may affect not only the attainment of height and sexual development, but also skeletal development. Endocrine diseases that may accelerate skeletal development include precocious puberty, adrenogenital syndrome, and hyperthyroidism.

Similarly, a general physical examination may show symptoms such as exophthalmos, virilisation of girls, acromegaly and gigantism, which are indicative of pathological

disorders and must also redirect the estimation of age. Another indication of a possible endocrine disorder is a discrepancy between skeletal age and dental age, as dental development normally remains unaffected by endocrine disorders (Schmeling et al. 2007).

#### 4. Bone age in carpus

The most studied anatomical region for age diagnosis, in particular before full maturity is reached at the age of 18, is the carpus and the hand (Garamendi & Landa, 2003).

Among the primitive series which analyzed epiphyseal maturation of the carpus as the main object of study or in the context of a general series of long bones are those by Stevenson in 1924, Galstaun G in 1930, Sidhom G and Derry DE in 1931, Borovansky L and Hnevkovsky O in 1929, Davies DA and Parsons FG in 1927, Paterson RS in 1929, Meenes TO and Holly LE in 1932, Adair FL and Scammon RE in 1921, Francis CC and Werle PB in 1939, and Pryor JW in papers published between 1908 and 1933. The latter author was among the first to note that ossification occurs earlier in females than in males, even during the foetal period.

However, it was not until large longitudinal population studies were carried out in the early 20<sup>th</sup> century under the auspices of private foundations in both Europe and the U.S. that the study of the carpus took a prominent role among the anatomical regions studied for age determination, in particular in adolescence (Bañón, 2004).

In 1937, Todd compiled hand radiographs taken of individuals during a study conducted by the Western Reserve University in Cleveland, Ohio, and together with comparisons made against the examination of a series of skeletons, he published his *Atlas of Skeletal Maturation* (Todd, 1937) based on the selection of a representative radiograph of the hand for each age and sex. The radiograph had to meet two requirements: have the same degree of development for the 28 bones and ossification nuclei which were the subject of the study, and be from an individual with a degree of body development which was within the average for their age and sex.

Todd's Atlas was used as a standard of reference until it was revised by WW Greulich and SI Pyle in 1950, who used it to compile their well known Atlas still widely used today, *Radiographic atlas of skeletal development of the hand and wrist*, published in two editions in 1951 and 1959 (Greulich & Pyle, 1950-1959). The Greulich and Pyle series was based on a total sample of 6,879 healthy middle-upper class North American children. Essentially, the method evaluates a "mean" bone age by matching an X-ray against the bones of a standard atlas, and normality estimates based on a range of results are made using standard deviation values.

During World War II the Oxford Health Survey was started and conducted by John Ryle on 470 children. The measurement data included radiographs of the carpus, which were analysed by Roy Acheson. Acheson looked to improve the Todd system by increasing the accuracy of the assessment. Under his proposal:

- Each bone or ossification nucleus was studied individually.
- Each bone or ossification nucleus was pre-assigned an identifiable stage as it matured, and each stage was given a progressive score.
- A final maturity score was calculated by adding up all of the partial scores obtained for each individual bone.

The skeletal maturity stage then yields a continuous value which can be used for growth measurements, such as height or weight.



Acheson's procedure was later refined by Tanner and Whitehouse into the Tanner-Whitehouse charts (Tanner et al, 1983). With this method, the study of each hand yields a total score which can be used to calculate an overall maturity stage which is then matched to distribution tables by age and sex based on percentile distributions.

Therefore, there are two main types of methods for bone age assessment based on carpal bones: atlas methods, with the Greulich and Pyle atlas being the main international standard of reference, and numerical methods, with the three editions of the Tanner-Whitehouse method being the main reference. There are some other mixed methods, such as the Thieman-Nitz method based on a German population. There are adaptations of these methods to virtually all populations in practically every country.

In principle it would appear that a numerical method such as the Tanner-Whitehouse method should be more reliable, however, in practice it is subject to intra- and interobserver errors similar to the GP method, and is negatively affected by technical problems arising from an incorrect positioning of the hand while the X-ray is taken, which can be better solved by graphical methods. Some authors recommend the application of the GP method instead of the TW3 for clinical purposes, on the basis of economy of means criteria after ascertaining that the TW3 method is far more costly in terms of time and the results are similar to those obtained through the GP method (Garamendi & Landa, 2003).

Several attempts have been made to develop TW2 and TW3 numerical system software for automated evaluation of X-ray plates. In theory, this would allow for a uniform quantification of results without interference from distortion factors derived from the observer. However, the results are not yet comparable to those obtained through the manual method, and collaboration between radiologists and IT experts is still needed to improve hardware and software systems.

The interpretation of bone age results obtained by any of the available methods must be adapted to the characteristics of the population of the subject of the study. The factors which may modify bone age progression in a particular subject are not perfectly defined, although several studies have identified differences associated with pathological, racial or socio-economic factors. The overall impression from the most recent research is that socio-economic factors, which affect the nutrition patterns and hygiene and health conditions of the subjects, are the most significant in terms of their capacity to modify results (Schmeling et al, 2000, 2001). Racial factors are discussed by several authors in different studies, and while they alone do not seem to justify significant differences in bone age, there are no unquestionable data allowing one to categorically affirm or deny the specific impact of this factor. On the contrary, the studies conducted are conclusive that certain pathological conditions may affect the results of bone age assessment, albeit the list of those pathological conditions cannot be considered in any way exhaustive.

There is a large number of studies in the context of ethnic and racial impact, some of them of questionable methodological basis and at times contradictory results, mainly conducted on populations of European Caucasians, North American Caucasians, other North American ethnic groups (including the genetically questionable racial group of Hispanics), different Mongoloid and Caucasian populations from Asia, and some incomplete studies on central and southern African Negroid populations.

The most recent studies in Europe appear to indicate that maturation rates for European Caucasians are close to those described in the GP and TW2 systems or are slightly delayed or advanced in relation to them. In some cases, the differences with the original methods were small yet so statistically significant that the need to create charts and atlases specific to these populations has been proposed.

Classical studies conducted in the seventies and eighties on Mongoloid Asian populations showed that the bone ages of the Chinese and Japanese groups were delayed in relation to chronological age during the prepubertal period; however, accelerated growth in the postpubertal period resulted in maturity being reached at a similar age as for European and American Caucasians. The most current series on modern populations with better socio-economic status show a trend to adjust results even further to the rate of bone age maturation of western populations. Similar findings have been reported in India and Pakistan, where studies have shown advances in bone age in relation to chronological age during the postpubertal period, more obvious in subjects from an upper social class.

In the U.S., studies indicate that Caucasian subjects either closely fit the GP and TW2 standards or often show a certain advance in maturation. By contrast, studies on Negroid subjects show contradictory results in the series. For Gross et al. the black race fits the GP standard better than the white race (Gross et al, 1995). In the series conducted by Ontell et al. and Lodler et al. the black race is advanced in relation to the GP standard (Lodler et al, 1993; Ontel et al, 1996). The series obtained by Marshall WA on black Jamaicans compared to the TW2 UK60 indicates delayed bone age from age 13 irrespective of socio-economic factors (Marshall et al, 1970). Lastly, in a study of black and white subjects in the U.S., Gilsanz V found no significant differences between bone age and chronological age in both races after the socio-economic factors were made equal (Gilsanz et al, 1988).

As far as our knowledge extends, the inhabitants of Muslim countries in the Near East and northern Africa, and populations of these countries who have migrated to developed countries have not been systematically studied and it is not known whether their rate of bone maturation is in keeping with the progression described for other populations (Souguir, 2002). The only studies available are those by Koc et al (Koc et al, 2001), Büken et al (Büken et al, 2007) and Garamendi et al (Garamendi et al., 2005). The study conducted by Koc A et al. on a modern Turkish population showed delayed bone age up until the age of 13, and a discreet advance after that age in relation to the GP atlas. A major objection to this study is that the population sample had a chronological age of only up to 17. This problem was later solved in a recent study reported by Büken et al., who examined carpal radiographs of 409 Turkish boys and girls of Caucasian background aged 11 to 19. Similarly to Koc et al., their study indicated that their population cohort exhibited advanced bone age between ages 13 and 17, and a relative delay in the 18 to 19 age group. In 2005, Garamendi et al. published a study of 114 Moroccan immigrants with a confirmed age of 12 to 25, presenting a joint analysis of the variations from the Greulich and Pyle standard for carpal X-rays as well as of the dental age assessed by orthopantomography.

Some authors consider that the socio-economic characteristics of each population are the most significant factors affecting variation in the rate of bone age maturation. Other studies, on the contrary, fail to confirm this hypothesis. A study on a black Jamaican population reported by Marshall WA found no variations according to the upper or lower social class of the subjects (Marshall et al., 1970). However, modern specific studies of this variable by authors such as Jahari AB et al on an Indonesian population (Jahari, 2000), Fleshman AK on an African population (Fleshman, 2000), and Melsen B et al on a population of adopted foreign minors in Denmark (Melsen et al, 1996) clearly identify socio-economic factors and poverty as causing significant delay in the rate of the bone maturation sequence during the prepubertal period.

Pathological factors clearly identified as altering bone age maturation rate include, among others, nocturnal enuresis (Dundaroz et al., 2001), GH deficit (Vallejo-Bolaños et al., 1999),

obesity (Bueno et al, 1996), high-level competitive sport activities (Theintz et al., 1993) or bone malformations, and exposure to physical agents causing injury such as frostbite (Freyshmidt et al., 2001).

## 5. Dental age

Dental age can be assessed accurately in childhood, because many of the teeth are developing simultaneously.

### 5.1 Dental eruption

Visual inspection of dental eruption was the first and most usual method for dental age assessment. In a work entitled “The Teeth, a Test of Age”, Edwin Saunders in 1837 proposed to the English parliament the use of the degree of dental eruption as a method to determine the age of child workers in factories, where the age limit was nine years (Bang, 1989). For a long time, and even up to the present day in many parts of the world where birth registers do not exist, dental development is used as a child age indicator, adopted as a biological-legal indicator.

However, although this method is fast, cheap and not very influenced by intra- and interobserver error, eruption is not a good age indicator when used alone, due to factors like interindividual or populational variation (Garn et al., 1959; Moorees et al, 1963), systemic or local diseases (Ungar, 1937) or the elapsed time without changes (Teivens & Mörnstad, 2001).

Numerous authors have investigated the chronology and sequence of eruption in different populations (Foti et al., 2003; Fulton & Price, 1954; Giles et al., 1963; Logan & Kronfield, 1933; Olze et al, 2007; Planells et al, 1993; Saunders et al 1993; Tanguay et al. 1984; Van der Linden, 1980). Some of this research examines the correlation between dental eruption and other development parameters; for example Lewis and Garn (Garn & Lewis, 1959) which evaluates parameters such as somatic and sexual growth, personality and state of health, or Green (Green, 1961) which attempts to establish correlations between dental, skeletal and chronological age and weight and height, finding a stronger correlation between dental and chronological age even than that existing between dental and bone development. Hagg and Taranger (Hagg & Taranger, 1980) study the relationship between dental eruption and maximum puberal growth, finding a low correlation between somatic and dental development. Baume and cols (Baume et al., 1954) have shown changes in dental eruption related to hypophyseal hormonal levels.

### 5.2 Dental maturation

Mineralization of deciduous tooth crowns begins at around 3 or 4 months of intrauterine life, with calcification continuing after birth during the neonatal period (Burdi, 1992). Root formation is generally completed between 18 months and 3 years of age.

Mineralization of adult teeth meanwhile takes around nine years, beginning with the first permanent molar around the moment of birth (Evans & Knight, 1981).

The dental development process correlates with different morphological stages of mineralization that can be observed with radiographic techniques, and undergo much more uniform and gradual changes than eruption; more controlled by genetics and less influenced by external factors than all other measurable criteria of maturity (Frucht et al., 2000). This is the reason why several methods of dental age assessment have been developed.

All the age assessment methods based on dental maturation follow the same procedure. First the stage of development of each of the teeth is evaluated from radiographic records, the method of choice being panoramic radiography or orthopantomography (OPT). Next the stage of development is related to the age of each tooth, derived from study of a sample of known age. This estimation method is based on subjective evaluation of the stages and has many defects. The biological variation in development is also wide for all teeth.

Due to the differences existing between methods and populations of different origin, these elements must be expressed as well as the confidence interval. Numerous studies have provided maturation scales in both deciduous and permanent teeth populations, identifying the successive states of development, though they show differences in the methodology employed (longitudinal versus transversal methods, definition of the development stages, etc) (Demirjian et al., 1973; Moorrees et al., 1963; Nolla, 1960). During the infant period where simultaneous development of several teeth can be observed the majority of these age assessment methods show variations of around 2 years to the average for confidence intervals of 90-95%, indicating rather low accuracy. Studies carried out on samples of known ages (Liversidge, 1994; Saunders et al., 1993), show differences of some 6 months to the real ages.

Nolla classified dental development into 10 calcificación stages from crypt state to closure of the root apex (Nolla, 1960). The Nolla study warned that mineralización development began and ended earlier in females, though there appear to be no differences in the sequence of development finalization. The Nolla method is one of the most widely used clinically as a reliable procedure for dental development estimation in permanent teeth. Diverse studies (Bolaños et al, 2003; Haavikko, 1974; Staaf et al, 1991), applying the Nolla method find an average assessment error of around 2 years for a 95% confidence interval.

One of the systems most universally used to evaluate the degree of permanent dental development is that proposed by Demirjian, Goldstein and Tanner (Demirjian et al., 1973), based on analysis of a sample of French-Canadian children. The original method evaluates the degree of calcification of the seven teeth in the left mandibular hemiarch, excluding the third molar, from radiographic records. 8 maturation stages (A to H) are established for each tooth, from the start of crown calcification to the root apex close, in a similar way to the Nolla method. Each tooth is attributed a formation stage, then converted into a score depending on the sex, following the same mathematical technique used to evaluate skeletal development by the Tanner-Whitehouse method (Tanner et al., 1975). The scores of the seven teeth are added to obtain the so-called dental maturity score on a scale of 0 to 100. This score is transformed through the corresponding tables into dental age. The method has the disadvantage that it does not include a valuation of the third molars, so can only be used for preadolescent ages.

Subsequently the same author has produced updates to the original method, proposing a valuation system for four teeth (both premolars and molars) with different standards (Demirjian, 1976).

In all cases, given that maturation development is different for the two sexes, the sex must be determined beforehand (Levesque et al., 1981).

The widespread use of this method as an infant age assessment procedure has meant that the results of the Demirjian study have been tested in other populations. Numerous studies over recent decades show a slight delay in maturation of the original French-Canadian population, causing overestimation when the original results of the method are applied to other populations (Bolaños et al., 2003; Davis & Hagg, 1994; Eid et al., 2002; Frucht et al.,



2000; Koshy & Tandom, 1998; Liversigde et al., 1999; Loevy & Goldberg, 1999; MacKenna et al., 2002; Nykanen et al., 1998; Nystro et al., 1986; Prabhakar et al., 2002; Staaf et al., 1991; Willems et al., 2001). The aforesaid overestimation varies between some months and several years in age, and it is recommended that standard values based on studies of the same population to which the method is applied are used.

Seeking a more accurate statistical model that explains as well as possible the correlation between the degree of dental development and chronological age, Teivens and Mönstard (Teivens & Mönstard, 2001) have recently produced diverse mathematical functions modifying the original Demirjian method, obtaining the best results when a cubic regression model is used ( $R^2 = 0.95$ ). This model has been tested in a comparative study between Swiss and Korean subjects, showing statistically significant differences, with earlier development in the Swedish sample of 2 months for males and 6 months for females.

### 5.3 The third molar in age assesment.

Age assessment becomes more complicated once the root apex of the second permanent molar has closed (at around 14 years of age) due to the variability of third molar development. The third molar is the tooth showing greatest frequency of agenesis (Garn & Lewis, 1962), the most irregular in its maturation sequence (Kieser, 1990) and, in contrast to the rest of dentition, tends to appear earlier in males than females (Levesque et al., 1981). However, due to the scarcity of alternative indicators maturity evaluation in this tooth is one of the prime tools in age assessment in these cases.

Recent years have seen a proliferation of studies focusing on third molar maturation as an age assessment method, with the purpose of contributing data enabling us to better understand the factors influencing the maturation of this tooth and establish more specific reference values providing more reliable diagnosis.

Despite this, the continual increase in immigration of young people from third world to industrialised countries, and the need to have a reliable and sound age assessment procedure in the absence of documents showing the date of birth, have for some years now prompted studies based on third molar development (Harris & Nortjé, 1984; Micci & Buzzanca, 1998; Robetti et al., 1982; Robetti et al., 1993). The third molar is the only tooth undergoing maturation during juvenile years, and is especially attractive as a study subject because the degree of mineralization can be easily ascertained through non-invasive methods such as radiology.

Of the numerous methods for third molar maturation evaluation, there seems to be a broad current consensus that the Demirjian method (Demirjian et al., 1973), is the most suitable, for several reasons:

The Demirjian stages are defined by morphological changes more objectively valued than speculative estimates of length (Olze et al., 2005, 2006). Stages of root formation are more clearly defined and show the highest values for inter-observer and intra-observer agreement and for correlation between the defined stages and true age (Dhanjal et al., 2006).

In comparison with other age diagnosis methods, those used in estimating legal age or minor status should minimise false positives with the aim of avoiding mistaken classification of a minor as of legal age.

Intra-observer and inter-observer agreement are reported to be high (Dhanjal et al., 2006; Orhan et al., 2007; Prieto et al., 2007) with best agreement for mandibular third molar when Demirjian's method is employed (Arany et al., 2004; Dhanjal et al., 2006).

In a study carried out by the Research Committee of the American Board of Forensic Odontology Mincer et al (Mincer et al., 1993) evaluate precision in age assessment from the



development state of the lower third molar, valued according to the Demirjian method. According to this study, stages A to D (up to complete crown formation) and stage H (complete apex closure) would show respectively a strong probability that the individual is younger or older than 18. Accuracy, established based on the difference between the real age and that calculated from the degree of dental development, is situated at 4.8 years for the 95% range (two standard deviations). Using the same valuation system in a Swedish population sample Thorson and Hägg (Thorson & Hägg, 1991) observed a weak relation between chronological and dental age (underestimation of chronological age), with a mean difference between the estimated and the chronological age of about  $\pm 4.5$  years in girls (95% confidence interval) and  $\pm 2.8$  years in boys, and intra-observer error of  $\pm 0.8$  years (95% confidence interval) which for the authors rules out this method for age assessment.

Kullman et al (Kullman et al., 1992) obtain similar results using a system based on classifying root development into 7 development stages, with standard deviations of 1 to 2 years to the average age in the different development stages.

In the review carried out by Rirz-Timme et al (Rotz Timme et al., 2000) the third molar gives standard error values (SEE) varying in a range of 1-2.5 years, with correlation ratios (r) between 0.32 and 0.85.

The use of standard error as a measure of accuracy has been criticised by diverse authors (Aykroyd et al., 1997; Giles & Klepinger, 1988) and recent papers propose the use of Bayesian probability as an alternative to regression analysis (Braga et al., 2005), though more studies with respect to these items are desirable.

Perhaps due to the fact that a good part of the third molar formation process occurs once puberty is reached, sexual dimorphism contrary to the rest of the dental maturation processes can be observed, with males reaching the different development stages earlier than females, independently of their ethnic origin. Studies published to date agree in this respect (Arany et al., 2004; Garn et al., 1959; Gunst et al., 2003; Haavikko, 1974; Harris, 2007; Harris & Nortjé, 1984; Köhler et al., 1994; Levesque et al., 1981; Meinl et al, 2007; Orhan et al., 2007; Prieto et al., 2007; Solari & Abramovitch, 2002; Throson & Hägg, 1991; Willerhausen et al., 2001).

Studies evaluating third molar development in the maxillary and mandibular arch appear to agree in the greater advance in maturation of maxillary molars versus mandibular ones (UNHCR, 2002; 6),(14),(37),Meinl et al., 2007; Orhan et al., 2007; Solari & Abramovitch, 2002; Willerhausen et al., 2001). For Mincer (Mincer et al., 1993) this could reflect different control mechanisms in the process of development of the two arches. He also observes that combining the results of the teeth of both arches seems to improve accuracy slightly.

Though impaction of the third molars has been put forward as a cause of delay in root formation (Köhler et al., 1994), a recent work by Friedrich (Friedrich et al., 2005) concludes that the topography of the wisdom teeth did not influence the timing of root development.

All studies coincide in the absence of significant differences depending on the side (left or right) (Meinl et al., 2007; Orhan et al., 2007; Prieto et al., 2007; Willerhausen et al., 2001).

Dental development can be altered by long term conditions, congenital syndromes, nutrition deficiencies or hormonal disorders, among others. Meanwhile, the factors influencing dental formation are difficult to identify.

Meinl et al (Meinl et al., 2007) call attention to the consequences of exclusion of subjects showing certain pathologies or irregular development, so that age assessment methods would be only applicable to individuals with a pretended normal dental state. In their study they decide not to carry out exclusions for such motives and include all the subjects of the

original sample, observing that none of the outliers had characteristics associated with altered growth, and also that individuals with severe development alterations did not show precarious findings. In accordance with these results they assume the hypothesis that dental development underlies strong regulation mechanisms which seem difficult to alter, even under pathological conditions.

As in all anthropological analysis, the characteristics of the reference population are a very significant element. The influence of genetic, nutritional, and geographical factors must be taken into account, when standards are developed. The applicability of standards generated to members of ethnic groups that are different from the reference population has been the subject of controversial discussion. Up to now several studies have been undertaken in different populations, with the aim of observing the usefulness of the third molar as a reliable indicator of age (Arany et al., 2004; Blankenship et al., 2007; Bolaños et al., 2003; Garamendi et al., 2005; Gunst et al., 2003; Gunst et al., 2003; Harris, 2007; Harris & Nortjé, 1984; Kullman, 1992; Martín, 2007; Meinel et al., 2007; Micci & Buzzanca, 1998; Mincer et al., 1993; Nambiar, 1996; Olze et al., 2004, 2006, 2007; Prieto et al. 2007; Robetti et al., 1982; Solari & Abramovitch, 2002; Illerhausen et al., 2001; Yaacob et al., 1996). These studies have proved that dental development shows slight variations among different populations, making specific studies necessary.

Although certain heterogeneity is observed when comparing the results of studies carried out on different populations, the results of a work (Prieto et al., 2007) performed on a sample of 1050 orthopantomographs of young Spaniards aged 14 to 21 show that the Spanish population undergoes maturation development of the lower third molars faster than the North American, French-Canadian, Austrian and Scandinavian populations, while more similar to the Hispanic population of the United States.

Garamendi et al (Garamendi et al., 2005) studied a sample of 114 illegal Moroccan immigrants, whose real age was subsequently obtained. The examination included radiographical dental study to estimate third molar maturation following the Demirjian and Goldstein method previously commented. The results show that this constitutes a good age diagnosis method – though the standards used were those obtained from the original French-Canadian population – while efficiency was increased by combining bone maturation valuation methods.

The lack of data on the influence of the ethnic factor in mineralization represents a restriction in the reliability of age assessment and therefore in the value of forensic information essential to legal soundness. This has come about for diverse reasons, such as the use of different assessment methods, small sample sizes, or African population birth data not checked, which prevent data being directly comparable.

In various studies together with South African and Japanese colleagues, Olze et al (Olze et al., 2004, 2006, 2007) evaluate the possible influence of the ethnic factor in third molar mineralization and eruption, observing a slower maturation process in the mongoloid population, which could reach the predominant stages of mineralization 1-2 years older and Africans 1-2 years younger, than Caucasoids and similar behaviour as regards third molar eruption. These authors think that these differences could be owed to the difference in the palate dimensions, smaller in mongoloids, which could cause a delay in eruption and because of this in mineralization.

Blankenship et al (Blankenship et al. 2007) and Harris (Harris, 2007) evaluate the differences between black and white North Americans. The results coincide with those of Olze et al. showing greater maturation speed in the black population.

The scientific studies performed, though representing an important contribution to our knowledge of third molar maturation, do not resolve the problem facing us in determining the age of a young illegal immigrant. In the case of Spain and the majority of European countries, these immigrants come in different proportions from Morocco, the sub-Sahara, Latin America and Eastern Europe.

Martín de las Heras et al (Martín, 2007) evaluates the development of the third molar in the northern Spanish population and the Spanish and Moroccan population resident in one of the Spanish cities of North Africa (Ceuta). The interest of this work centres on the fact that the Maghreb population is one of the most frequent illegal immigrant groups in Spain and other Mediterranean European countries.

The lack of maturation standards for African countries and the difficulty of carrying out studies in the countries of origin makes it necessary to perform studies enabling us to evaluate dental maturation in these countries to arrive at more reliable data.

The inter-population differences shown by these studies underline the need for specific work in this area. More research is needed in this field.

The results of the work published agree in stating that once the third molar reaches the H stage of Demirjian, the probability that an individual is of legal age is greater than 90%, independently of ethnic origin, sex or the tooth evaluated (Arany et al., 2004; Meinl et al., 2007; Mincer et al, 1993; Solari & Abramovitch, 2002). For this reason this element can be considered a useful marker to resolve the question of whether an individual of unknown age can be considered an adult for legal effects.

Only in criminal cases is the use of radiography justifiable.

In practice it is not habitual to request the express consent of a minor to carry out the exploration and complementary examinations, including radiological tests. Given that the examination will affect their privacy and that radiological tests mean exposure to radiation with potentially harmful effects, as we understand it it should be obligatory to obtain the consent of the subject, and to suspend examination in the absence of consent. This aspect is included in the guide of good practice of the program for unaccompanied minors in Europe. It must further be appreciated that using techniques developed for clinical purposes - to seek possible pathologies - in evaluating the degree of maturation for a specific age represents a perversion, as it inverts the application in the search for evidence to deduce the age of an individual from their maturation level when there is no clinical reason justifying such use.

Attempts to standardisation, calibration and evaluation procedures have been scarce up to now, pointing to the need for guidelines on this and all other aspects.

The difficulty involved hence in diagnosis and the potential sources of evaluation makes it necessary to draw up common guidelines for action, based on scientific evidence and unifying the criteria to be followed, such as those of the Study Group on Forensic Age Diagnosis (AGFAD), founded in Berlin in 2000. The group has published guidelines on age diagnosis on living individuals for criminal, civil and asylum proceedings among others (Schmeling et al., 2007).

The International Organization for Forensic Odonto-Stomatology (IOFOS) has published recommended procedures for quality assurance in forensic dental age estimation <http://www.odont.uio.no/foreninger/iofos/quality/Age-IOFOS.htm>

Regarding these recommendations, Solheim and Vonen(86) highlight the discrepancies of criteria among experts over the steps to follow between those who only wish to apply a statistical method and report on the results and those who prefer to express the expert

opinion taking into consideration the life conditions of the individual such as state of health and nutrition, clinical findings and of course the results of the statistical scientific methods. These authors coincide with the German Interdisciplinary Working Group for Age Diagnostic (34) that the conclusions should end with a complete assessment of the most likely chronological age.

Just as Ritz-Timme et al (Ritz-Timme et al., 2000) recommend, efforts should be made to develop external quality control, something perfectly possible in the field of age assessment, with the aim of guaranteeing quality standards enabling an adequate response given the important role played by forensic medicine in the legal and social fields of age assessment.

#### **5.4 Age estimation in the clavicle**

To answer the question of whether a person has reached the age of 18 it is particularly helpful to evaluate the ossification status of the medial epiphysis of the clavicle, because all other examined developmental systems may already have completed their growth by that age.

A number of studies have been conducted on the time frame for the ossification of the medial clavicular epiphyseal cartilage in the age group concerned for forensic age diagnostics in living individuals. One group of studies adopted an anatomical perspective, assessing ossification by means of autopsy or direct skeletal inspection (Todd & D'Errico, 1928; McKern & Stewart, 1957; Owings Webb & Myers Suchey, 1985; MacLaughlin, 1990; Ji et al., 1994; Black & Scheuer, 1996; Shirley, 2009; Singh & Chavali, 2011), while the other group took a radiological approach.

Several authors pointed out, that data from dry bone material are not directly comparable with data from radiological studies. Krogman and Isçan (Krogman & Isçan, 1986) as well as Kreitner et al. (Kreitner et al, 1998), for example, argued that commencement of fusion can be detected in radiographs before any union of epiphysis and metaphysis can be visible on dry bone. Therefore reference values from dry bone studies should not be applied to assessments based on radiographs.

Radiological methods to examine the medial clavicular epiphysis in living individuals are conventional radiography (CR) (Flecker, 1933; Galstaun, 1937; Jit & Kullkarni, 1976; Schmeling et al., 2004; Garamendi et al. 2011), computed tomography (CT) (Kreitner et al., 1997, 1998; Schulz et al., 2005; Schulze et al., 2006; Bassed et al., 2010; Kellinghaus et al., 2010a, b), as well as new approaches using magnet resonance imaging (Schmidt et al., 2007; Hillewig et al., 2011) and ultrasoundsonography (Schulz et al., 2008b; Quirmbach et al., 2009; Schulz et al., 2010).

While traditional classification systems differentiate between four stages of clavicle ossification (stage 1: ossification centre not ossified; stage 2: ossification centre ossified, epiphyseal plate not ossified; stage 3: epiphyseal plate partly ossified; stage 4: epiphyseal plate fully ossified), Schmeling et al. (Schmeling et al., 2004) divided the stage of total epiphyseal fusion into two additional stages (stage 4: epiphyseal plate fully ossified, epiphyseal scar visible; stage 5: epiphyseal plate fully ossified, epiphyseal scar no longer visible). Figure 1 shows the stages of clavicular ossification for CR and CT.

There is only one study referring to conventional radiography that meets the requirements of a reference study as stated by the Study Group on Forensic Age Diagnostics (Schmeling et al., 2008). In this study the earliest age at which stage 3 was detected in either sex was 16 years. Stage 4 was first observed in women at 20 years and in men at 21 years. Stage 5 was first achieved by both sexes at age 26 (Schmeling et al., 2004). It was concluded that plain



chest radiographs can essentially provide a basis for assessing clavicular ossification. If overlap in posterior-anterior views impedes evaluation additional oblique images (Bontrager & Lampignano, 2009) should be taken to facilitate age estimation.

In 1997 and 1998, Kreitner et al. published the first CT-based studies in which the medial epiphyseal ossification of the clavicle was evaluated applying a four stage scheme. Since these studies did not discriminate results by sex, their forensic value is limited. In a CT study conducted by Schulz et al. in 2005, presenting more cases and results discriminated by sex, the five stage classification by Schmeling et al. (Schmeling et al., 2004) was used. The earliest occurrence of stage 3 in females was noted at age 16 and in males at age 17. Stage 4 was first achieved by both sexes at age 21. Stage 5 was first noted in females at age 21 and in males at age 22, which is 4 or 5 years earlier than is reported in the conventional radiographic studies. Schulz et al. (Schulz et al., 2005) raised the question whether CT scans with a slice thickness of > 1 mm could cause misinterpretation of clavicle ossification status and recommended examining the influence of slice thickness on the age intervals of ossification stages in additional studies.

In a study on the influence of the slice thickness on the ability to assess the stages of clavicular ossification Mühler et al. (Mühler et al., 2006) retrospectively analysed the CTs of 40 individuals which have been examined within the scope of age diagnostics. Scans with slice thicknesses of 1, 3, 5, and 7 mm have been reconstructed from the obtained data. Seven out of 80 clavicular epiphyseal plates showed differences depending on the slice thickness in the particular stages of ossification. In 1 case a slice thickness of 1 mm led to a different diagnosis of the ossification stage than a slice thickness of 3 mm, in 3 cases the diagnoses differed between the slice thicknesses of 3 mm and of 5 mm, and in another 3 cases between 5 and 7 mm. The authors therefore concluded that for age estimation purposes the slice thickness should be 1 mm in order to ensure maximum accuracy and diagnostic reliability. The findings of this study were confirmed by Kaur et al. (Kaur et al., 2010).

Recently, Kellinghaus et al. (Kellinghaus et al., 2010) published data from a thin-slice CT study. In this study stage 3 was first achieved by male individuals at age 17 and in females at age 16. The occurrence of stage 4 was first found in both sexes at the age of 21. In either sex, the earliest observation of stage 5 was at age 26. These findings are consistent with the data from the conventional study of the clavicle (Schmeling et al., 2004).

A further improvement of age diagnostics based on clavicular ossification was the subdivision of stages 2 and 3 by Kellinghaus et al. (Kellinghaus et al., 2010). The sub-classification stages were defined as follows (see figure 2):

- Stage 2a: The lengthwise epiphyseal measurement is one third or less compared to the widthwise measurement of the metaphyseal ending
- Stage 2b: The lengthwise epiphyseal measurement is over one third until two thirds compared to the widthwise measurement of the metaphyseal ending
- Stage 2c: The lengthwise epiphyseal measurement is over two thirds compared to the widthwise measurement of the metaphyseal ending
- Stage 3a: The epiphyseal-metaphyseal fusion completes one third or less of the former gap between epiphysis and metaphysis
- Stage 3b: The epiphyseal-metaphyseal fusion completes over one third until two thirds of the former gap between epiphysis and metaphysis
- Stage 3c: The epiphyseal-metaphyseal fusion completes over two thirds of the former gap between epiphysis and metaphysis



Stage 3c first appeared at age 19 in both sexes. If stage 3c is found, it is therefore possible to substantiate that an individual has already reached the legally important age threshold of 18 years.

For forensic age estimations in living individuals, non-ionising procedures for the presentation of the medial clavicular epiphyseal cartilage would be desirable, as the radiation exposure from the necessary imaging examination could be decreased considerably. Against this background Schmidt et al. (Schmidt et al., 2007) prospectively evaluated magnetic resonance (MR) scans of 54 sternoclavicular joints of bodies aged between 6 and 40 years. All of the examined medial clavicular epiphyseal cartilages permitted an assessment of the degree of ossification. Stage 2 was first observed at the age of 15 years, the earliest age at which stage 3 was observed was 16 years, and stage 4 was first observed at the age of 23 years. Very recently Hillewig et al. (Hillewig et al., 2011) published a four-minute approach for MRI of the medial clavicular epiphysis in living individuals.

In a comparative study using CR, CT and MRI for staging of 15 sternoclavicular joints the ossification stage was in agreement in each of the three imaging methods used in 6 cases (Vieth et al., 2010). In the remaining cases the ossification stage was assessed either one stage higher or lower in one of the applied imaging methods than in the other two techniques. In five cases (compared to CT), respectively seven cases (compared to MR), CR showed a higher ossification stage than CT and MR imaging. In two cases CR resulted in the determination of a lower stage than in CT and MR imaging. Twice the MR scans showed a less advanced ossification stage than CT-based images. The authors concluded that in age estimation practice, modality-specific reference studies are to be applied in order to guarantee an adequate assessment of the ossification stage of the medial clavicular epiphysis.

Another radiation free approach to evaluate the ossification stage of the medial clavicular epiphyses is ultrasound sonography. Schulz et al. (Schulz et al., 2008) prospectively evaluated 84 right clavicles of test subjects 12–30 years of age by means of ultrasound. For the sonographic assessment of the clavicle ossification, the traditional classification had to be modified as follows (see figure 3):

Stage 1: The medial end of the clavicle is configured acute-angled. A bony center of ossification is not representable.

Stage 2: The medial end of the clavicle is separated from the bony center of ossification by a sound gap.

Stage 3: Both an ultrasound gap with a bony center of ossification and a fully ossified epiphyseal plate with a convex curved end of the clavicle are representable.

Stage 4: The medial end of the clavicle is convex curved. A bony center of ossification is not representable.

The earliest ages at which the respective ossification stages were observed were 17 years for stage 2, 16 years for stage 3, and 22 years for stage 4. Another pilot study by means of sonography was performed by Quirnbach et al. (Quirnbach et al., 2009). In this study stage 4 was first observed at 20 years. These results were reviewed in a reference sample of 601 healthy volunteers aged between 10 and 25 years by Schulz et al. (Schulz et al., 2010). The earliest observation of stage 4 in women was at 19 years in both sexes. Based on these findings it can reliably be stated that an individual with stage 4 has already accomplished 18 years of age.

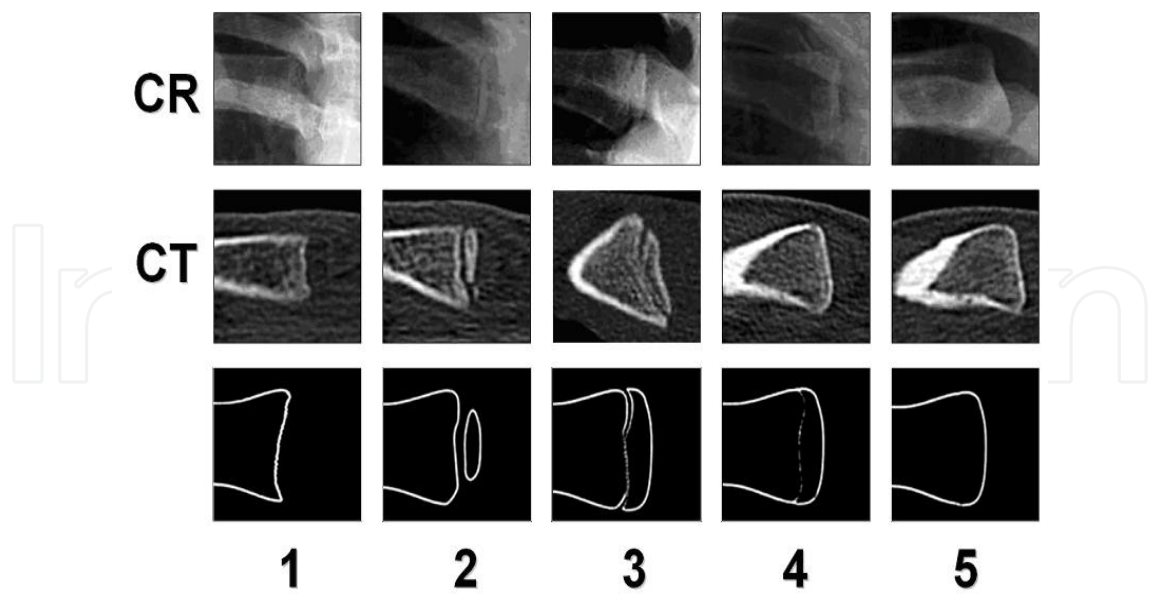


Fig. 1. Schematic drawings and pictures of the stages 1-5 of clavicular ossification as revealed by conventional radiography (CR) and computed tomography (CT)

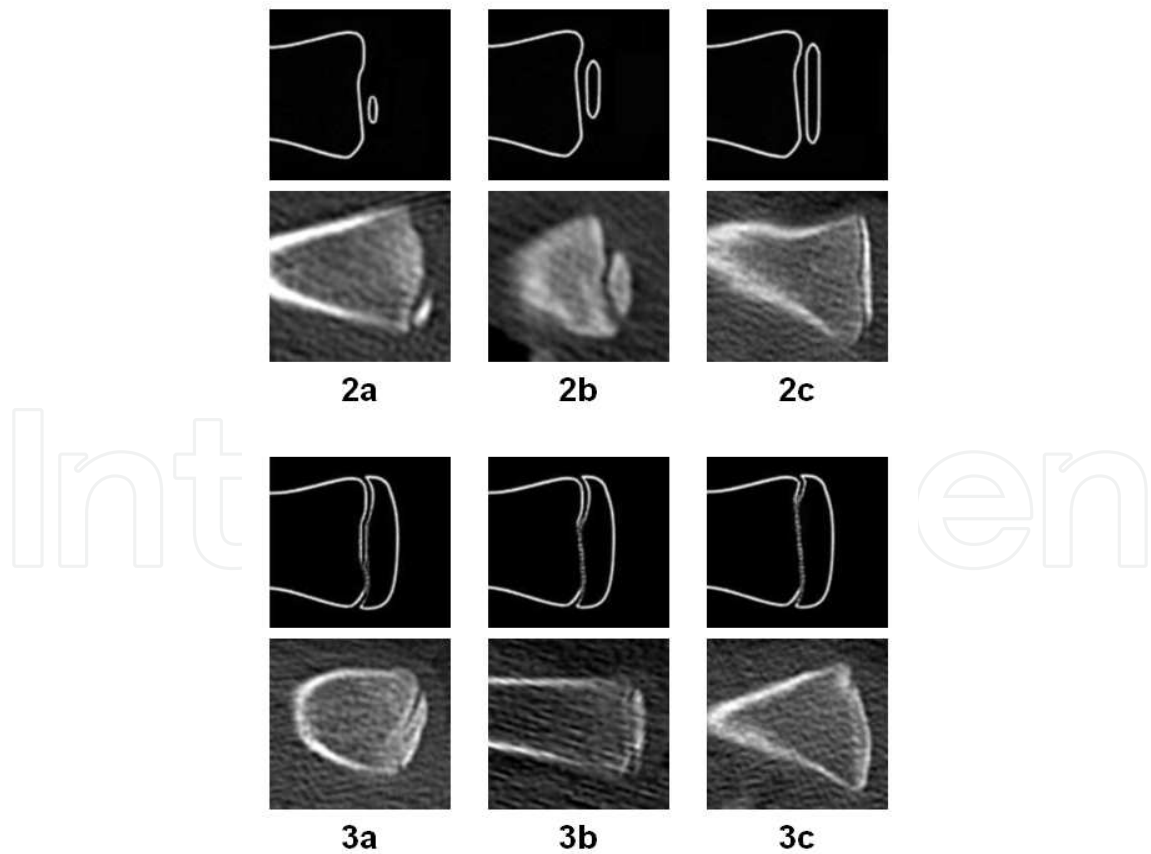


Fig. 2. Schematic drawings and pictures of the stages 2a-3c of clavicular ossification as revealed by means of thin-slice CT

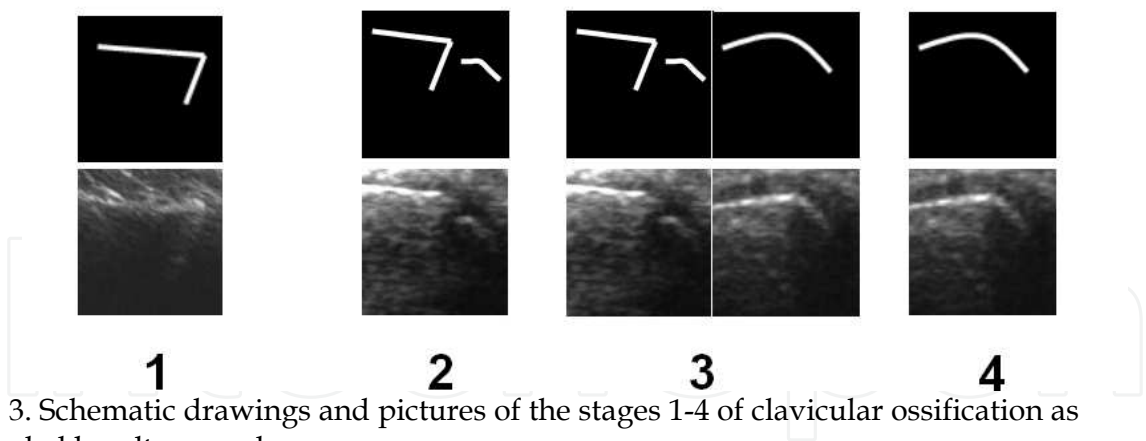


Fig. 3. Schematic drawings and pictures of the stages 1-4 of clavicular ossification as revealed by ultrasound

6. Interpretation of results

6.1 Interpretation of results from combined methods. The clavicle and the twilight zone

AGFAD Guidelines on Age Estimation in Living Subjects recommend that when performing a FAE different methods must applied in the same subject. These recommendations mean that in the same subject different FAE results will be available (AGFAD, 2001).

Previous series (Garamendi et al., 2005) proved that the accuracy of FAE improves when different age estimation methods are simultaneously applied. The under ROC curve area increase when both bone age and dental age methods are applied when assessing if a subject could be older or younger than 18.

In cases of combined analysis of age estimation based on dental age and bone age in carpus, the lowest result of both methods should be used for a final estimation because of ethical reasons and legal accuracy.

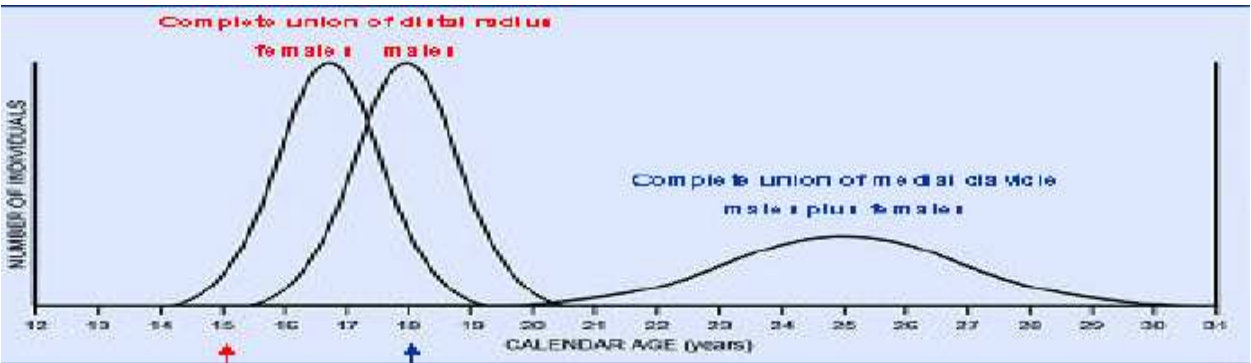


Fig. 4. Simulacra of distribution of results of bone age in carpus and in clavicle in relation with complete fusion of distal radius and complete union of medial clavicle.

More complex is the application of results in case of doubt about an age estimation over or under 18 years or 21 years old based on results of an age estimation in the clavicle. Positive results (clavicle stage 4 of Schmeling’s method) indicate positively that the age of the subject will be most probably older than 21,6 in males and 21,3 in females (Kellinghaus et al., 2010). Nevertheless, stage 3 is observed in subjects up to 26 years old and aritmetical mean of stage 4 is 29,6 +/- 4,2 in males and 8,2+/-4,2 in females. 100% of the sample in stages 4 or 5 of

Schmeling method is observed only in subjects older than 26 (Kreitner et al., 1997; Schmeling et al., 2004). These statistical results are graphically explained in figure 4 in which it's clear that a negative result of stage 4 in a subject older than 21 years and younger than 26 is in many cases most probable than a positive result. It turns the range between 18 and 26 years old a twilight zone in which a positive result is definitive but a negative result is the most probable one and suppose the impossibility to assess a FAE of being older than 21 years old in most cases of subjects younger than 26.

Nevertheless, more research is needed in this specific field to define more precisely the best standardized approach to FAE based on simultaneous different methods.

## **6.2 Ethnical and socioeconomic factors: sexual maturation, ossification, dental development**

Since the subjects whose age has to be estimated mostly belong to populations for which no reference studies are available that could be used for forensic purposes, the question arises whether there are significant developmental differences between various ethnic groups which would prohibit the application of relevant age standards to members of ethnic groups other than the reference population. In this respect the term 'ethnicity' shall be used only to identify the affinity of various populations in terms of origin.

Comprehensive studies of the relevant literature revealed that the major ethnic groups of interest to forensic age estimation achieve defined stages of ossification, dentition and sexual maturity in the same natural sequence, so that it is generally possible to apply the relevant reference studies also to other ethnic groups (Schmeling et al., 2001).

### **6.2.1 Sexual maturation**

Data on sexual maturation is relatively scarce. Comparative studies on sexual maturation were conducted by Harlan et al. (Harlan et al., 1979, 1980), Channing-Pearce and Solomon (Channing-Pearce & Solomon, 1987), Wong et al. (Wong et al., 1996) and Huen et al. (Huen et al., 1997). Between 1966 and 1970, Harlan et al. (Harlan et al., 1979) analysed the sexual development of 6,768 male Americans aged between 12 and 17 years. They found no significant differences between blacks and whites. In 1980 Harlan et al. (Harlan et al., 1980) published a representative study examining the sexual maturation of a female American population of the same age group. This study observed relatively faster rates of maturation for blacks compared to whites. Channing-Pearce and Solomon (Channing-Pearce & Solomon, 1987) examined sexual development in a study involving 362 black and 355 white girls in Johannesburg, South Africa. Unlike Harlan et al. (Harlan et al., 1980), they came to the conclusion that black girls on average reached full sexual maturity later than white girls. Wong et al. (Wong et al., 1996) examined sexual maturation in a 1993 study involving 3,872 boys from southern China. They found that the time pattern of sexual maturation was comparable to that of Europeans, with the exception that Asians developed pubic hair later. Huen et al. (Huen et al., 1997) published a similar study including 3,749 girls from southern China. They found that, according to the mean values for the individual stages of maturity, the examined girls were among the earliest to reach sexual maturity worldwide.

### **6.2.2 Ossification**

Numerous studies are available on skeletal maturation of all major ethnic groups (Africans, Australians, Caucasoids and Mongoloids) (Schmeling et al., 2000a). Because there are



several potential factors of influence and their simultaneous action makes assessment of population differences a difficult exercise, all the more as the validity of some of those investigations seems to be limited to small sample size, the exclusive consideration of non-relevant age groups, lack of information on health, ethnic identity and socioeconomic status and absence of confirmed data on proband age. Hence, for the problem at hand, greatest relevance may be claimed for studies on various ethnic groups of similar socioeconomic status and living in one and the same region or populations of one and the same socioeconomic status living in different regions. Such studies are available from the USA where research has been conducted on descendants of Caucasoids, Mongoloids and Africans as well as from numerous ethnic groups of the former Soviet Union.

In a comparison with the Greulich-Pyle standards, Sutow (Sutow, 1953) discussed racial differences as one of the causes of retarded skeletal maturation of Japanese children living in Japan. His findings were checked by Greulich (Greulich, 1957) who referred to Japanese individuals living in the USA. He studied hand bone development in 898 children of Japanese descent aged between 5 and 18 years living in the San Francisco Bay area of California. While retarded skeletal maturation, in comparison with the Greulich-Pyle standards was recorded by Sutow for all age groups of Japanese living in their own country, such retardation was detected by Greulich only in boys aged between 5 and 7 years. Boys aged between 13 and 17 and girls between 10 and 17 years even exhibited comparative acceleration. Greulich concluded that the significant retardation, in comparison with the Greulich-Pyle standards, recorded for children living in Japan was attributable to less favourable nutritional and environmental conditions rather than to racial differences. Improved living standards in recent decades resulted in accelerated skeletal maturation even in Japanese living in Japan (Kimura, 1977a, 1977b) which, in the meantime has come to lie within the range of socioeconomically advanced European populations (Beunen et al., 1990; Wenzel et al., 1984).

Whereas some authors (Massé & Hunt, 1963; Garn et al., 1972) reported comparatively accelerated skeletal development in Africans in early childhood, ethnic origin obviously has no significant impact on the bone growth rate in later childhood and adolescence. Platt (Platt, 1956) studied skeletal maturation in 100 black inhabitants of Florida, 143 blacks in Philadelphia and 100 whites in Philadelphia aged between 5 and 14 years. In none of these three groups was skeletal age, as determined by identical X-ray standards, significantly different from chronological age. Platt compared his results with studies on black residents of Africa. Mackay (Mackay, 1952) recorded retardation by 1.5 to 2 years for East Africans, while Weiner and Thambipillai (Weiner & Thambipillai, 1952) recorded an average retardation of 16 months for West Africans. The assumption of an ethnic impact on skeletal maturation would justify expectation of a continuous series of phenomena ranging from severe retardation in blacks in Africa to moderate retardation in black Americans who had mixed with whites to absence of retardation in whites. Such continuous series do not exist, and consequently Platt, postulated that health and nutrition are the major factors influencing skeletal maturation.

Skeletal maturation in 461 black and 380 white Americans in the Lake Erie region was studied by Loder et al. (1993) between 1986 and 1990. Using the atlas method of Greulich and Pyle on the age group of 13-18 years, they recorded comparative acceleration of 0.45 years for white boys, 0.16 years for white girls, 0.38 years for black boys and 0.52 years for black girls. Johnston (Johnston, 1963) studied the same age group of white Americans in



Philadelphia by the same method and found acceleration values of 0.39 years for boys and 0.58 years for girls. Johnston's data for white Americans were almost identical with Loder's findings for black Americans, which seems to clearly underline that in the populations of the age group studied there were no ethnic differences with regard to skeletal maturation. Roche et al. (Roche et al., 1975, 1978) investigated skeletal maturation in the context of race, geographic region, family income and educational standards of parents in a representative cross-section of the US population aged between 6 and 17 years. They found no consistent black-white differences, no significant differences between regions and no urban-rural differences.

Comprehensive studies were conducted on skeletal maturation in different ethnic groups of the former Soviet Union, and 16 studies of 17 ethnic groups in different climatic and geographic zones of the former Soviet Union were evaluated by Pashkova and Burov (Pashkova & Burov, 1980). Included were Russians, Ukrainians, Georgians, Armenians, Azerbaidjanis, Balkarians, Cabardines, Kazakhs, Tadchiks, Uzbeks, Ingushi, Chechenians, Udmurtians, Chukchen, Koryaks, Intelmenians and Evenkians. The range of variation at all stages of skeletal maturation was less than one year in all populations studied. However, the causes of those variations were attributed by the authors to relatively small samples, different methods and techniques used in the studies or undiagnosed clinical conditions of probands but were not attributed to ethnic, regional or climatic differences.

Studies evaluated so far seem to suggest that there is a genetically determined element to skeletal maturation which does not appear to depend on ethnicity and may be exploited under optimum environmental conditions (i.e. high socioeconomic status), whereas a less favourable environment may lead to retardation of skeletal maturation. Applying X-ray standards to individuals of a socioeconomic status lower than that of the reference population, usually leads to underestimating a person's age. In terms of criminal responsibility, this has no adverse effect on the person concerned (Schmelting et al., 2000, 2006).

### 6.2.3 Dental development

Few comparative studies are available on the subject of third molar mineralization. Gorgani et al. (Gorgani et al., 1990) examined 229 black and 221 white US citizens aged 6-14 years. Among the black subjects crown mineralization of the third molars was completed 1 year earlier. Harris and McKee (Harris & McKee, 1990) studied 655 white and 335 black US citizens aged 3.5-13 years. Whereas the black subjects reached the earlier stages of third molar mineralization about 1 year earlier, the gap appeared to narrow for later stages. This trend is confirmed by the work of Mincer et al. (Mincer et al., 1993). They examined 823 US citizens (80% white, 19% black) aged 14-25 years but did not establish any significant differences in the time frame for third molar mineralization. Daito et al. (Daito et al., 1992) addressed third molar mineralization in 9111 Japanese children aged 7-16 years and compared their data with the values provided by Gravely (Gravely, 1965), Rantanen (Rantanen, 1967) and Haavikko (Haavikko, 1970) for Caucasoid populations. No significant differences were discovered. These studies only lend themselves to limited comparison due to small sample sizes, varying methods and assessment by different observers. A further problem lies in the fact that the age data for subjects of black African origin often was not verified. Moreover, most available studies focus on the earlier stages of mineralization.

A comparative study of third molar mineralization (Olze et al., 2004) was carried out on three population samples: one German, one Japanese and one South African. To this end,

3652 conventional orthopantomograms were evaluated on the basis of Demirjian's stages. Statistically significant differences between the samples investigated were established for the age at which stages D-G of third molar mineralization were achieved. Significant differences between German and Japanese males were noted for stages D-G of mineralization. Significant differences between Japanese and German females were observed for stages D-F. According to these findings, Japanese males and females were approximately 1-2 years older than their German counterparts when they reached stages D-F. Significant age differences between South African and German males applied to stages D-E. Significant age differences between South African and German females were observed for stages E and G. The South African subjects were approximately 1-2 years younger than the German subjects upon achieving these stages of mineralization. Significant age differences between the South African and Japanese samples were ascertained for both sexes at stages D-G. The South African subjects were approx. 1-4 years younger than the Japanese subjects upon reaching these stages.

The population differences observed here may be due to differences in palatal dimensions between the ethnic groups surveyed. The largest palatal dimensions are observed in Africans and the smallest in Mongoloids, with Caucasoids assuming the middle rank (Byers et al., 1997). Inadequate space in the maxillary crest causes delay in third molar eruption, if not retention (Fanning, 1962). In turn, retained third molars mineralize later than teeth whose eruption has not been impeded (Köhler et al., 1994). This would explain why Caucasoid populations occupy the middle position in relative terms when it comes to third molar mineralization, while Mongoloid populations display a comparative delay and African populations a relative acceleration.

With regard to the eruption of third molars, some studies have found significant differences between specific populations (Schmeling et al., 2001). While in Caucasian populations third molars generally do not erupt before age 17 (Müller, 1983), Brown (Brown, 1978), Chagula (Chagula, 1960), Otuyemi et al. (Otuyemi et al., 1997), and Shourie (Shourie, 1946) describe cases of eruption starting in African, Australian and Indian populations already at age 13.

Comparative studies on the relation between age and third molar eruption are available for black and white Americans, Africans, and Asians. Garn et al. (Garn et al., 1972) studied the dentition of all permanent teeth in 953 black and 998 white Americans. In black Americans, the maxillary third molars developed 3.7 years earlier, and the mandibular third molars 5.6 years earlier, than in white Americans. Hassanali (Hassanali, 1985) compared the eruption times of third molars in 1,343 Africans and 1,092 Asians in Kenya. He found that in Africans third molars appeared two to three years earlier. The forensic applicability of these studies is limited, since age data for subjects of African origin are often not verified.

Olze et al. (Olze et al., 2007) analyzed and compared the chronological course of third molar eruption in German, Japanese, and South African populations with proven age of subjects. They found that their German sample had an intermediate rate of dental development as determined by comparing the different ages of third molar eruption. The defined eruption stages occurred at earlier ages in the investigated South African sample, and at later ages in the Japanese sample. Statistically significant population differences were observed in males at stages A and B. The South African males were on average 3.0 to 3.2 years younger than the German males at these stages of development, and the Japanese males were on average 3.1 to 4.2 years older than their South African counterparts for the same developmental stage. The females exhibited statistically significant population differences at stages A, B and C. The South African females reached the target stages on average 1.6 to 1.8 years earlier

than the German females, whereas the Japanese females were on average 0.9 to 3.3 years older than their German counterparts. It was concluded that population-specific reference data should be used when evaluating third molar eruption for the purpose of forensic age estimation.

### **6.3 Pathological factors that produce bone age retardation and acceleration**

Pathological conditions alter FAE based on methods of bone and dental age maturation. Albeit being less affected by pathological and ambient conditions than bone age, biomedical literature proves that dental age can be altered by some entities not necessary pathological, like delayed puberty or obesity.

It has been repeatedly demonstrated in the literature that bone age is affected by a wide range of pathological conditions. Main pathological conditions altering bone age maturation are those endocrinological conditions that modify the hypothalamus - hypophysis- gonads axis. Nevertheless other pathological entities affect bone age maturation: some clinical syndromes (as Soto's syndrome or Weaver's syndrome), bone dysplasias (some of them accelerating and other decelerating bone age maturation) and some drugs intake (table 1 and 2) (Taybi & Lachman.1990).

Forensic examiner must be aware of these conditions. The existence of such pathological conditions or drugs intake must be elucidated, as they evidently affect the bone age and dental age maturation. The results of a FAE must include corrections in relation with the existence of such accelerating or decelerating factors.

## **7. Ethical questions**

### **7.1 Exposition to radiation without clinical indication**

When an X-ray examination is carried out exclusively with FAE purposes and without a clinical indication the question arises on the possibility of detrimental effects due to the radiation exposure (European Commission 2004)

The effective dose from an standard X-ray examination of the hands is 0,1 microSievert ( $\mu\text{Sv}$ ), 26  $\mu\text{Sv}$  in case of Orthopantomograms, 220  $\mu\text{Sv}$  in X-ray examination of the proximal epiphyses of the clavicle and 600 to 800  $\mu\text{Sv}$  in case of TC of the sternoclavicular joints. The effective dose in case of a complete thorax TC is 6,6 mSv (Rammstahler et al.2009).

Some authors indicate that an amount of effective annual doses for X-ray examinations of less than 10  $\mu\text{Sv}$  are negligible. Other authors have stressed the insignificance of these usual FAE examination doses in comparison with naturally-occurring and civilizing radiation exposure. The effective dose from naturally-occurring radiation exposure has been calculated as an average in Germany at about 1,2 mSv per year and in The Netherlands at about 2,0 mSv. Fly staff of airplanes receive an average of 2000 mSv per year as a result of staying high in air (cosmic radiation). The radiation exposure from intercontinental flight at an altitude of 12000 meters is 0,008 mSv per hour (Schmeling.2008).

It follows that the radiation dose effective in case of an intercontinental flight is equivalent to 2 orthopantomograms and a CT of sternoclavicular joints is equivalent to 3,5 months of naturally occurring radiation exposure. On the basis of these comparisons the health risk as a result of usual X-ray examinations for FAE is negligible (Schmeling.2008)

Nevertheless, radiation exposure produce not only stochastic but non-stochastic damages the physicians must be aware of. Non-stochastic effects appear above 100 mSv and are therefore irrelevant in usual radiological diagnosis. But non-stochastic effects don't have

such a threshold and are not dose related, so their eventual appearance in case of X-rays examinations must be cautiously considered. Some authors minimize and other maximize the harm inherent to these non-stochastic effects (Garamendi&Landa.2010)

Bone Age Advanced		Drugs altering bone age
Adrenal Hiperplasia	Gigantism	Amiodarone
Acrodysostosis	Hypertiroidism	Budenoside
Adrenocortical Tumour	Idiopatic isosexual precocious puberty	Buclometasone, Dipropionate
Aldosteronism, primary	Lipodistrophy	Phenitoine
Arthrogryposis	Marshall Smith Syndrome	Metilphenidate
Beckwith-Wiedemann, syndrome	McCune Albright Syndrome	
Cerebral tumour	Neurofibromatosis	
Cockayne syndrome	Obesity	
Congenital Adrenal Hyperplasia	Peripheral dystosis (Brailsford)	
Cushing Syndrome	Sotos syndrome	
Ectopic Gonadotropine Production	Trisomy 8 Syndrome	
Familar Advanced Bone Age	Weaver syndrome	

Table 1. Pathologic factors associated with bine age acceleration and drugs intake that alter bone age process.

Anyway, albeit apparently negligible the possible detrimental effects due to radiation exposure must be considered. A best practice in FAE on the use of X-ray exams without other medical indications should avoid unnecessary repetition of the exams to minimize the radiation dose effective received by probands, should include the practice of the minimum necessary exams to avoid unnecessary exams and should include the elaboration of National Registers of unaccompanied minors to avoid the unnecessary repetition of all tests by different physicians at different dates (Garamendi et al.2011)

7.2 Ethical dimension of the expert report

Forensic physicians involved in FAE case analysis must be conscious of the ethical dimension of the conclusions written on their expert reports. Dr. Mata in 1842 pointed out that expressing an expert opinion in a Justice Court must be cautious (Mata, 1842). This old professor reminds future forensic physicians that writing a report expressing complete reliability in a question without a solid scientific basis could be an error. Some physicians understand that expressing themselves with complete certainty at Justice Court helps judges to decide on questions like FAE more clearly. Oppositely, this is not an advisable attitude as it can give the Court a false impression of certainty in questions not completely certain in the state of the art for the scientific community. This very same principle is the one precluded by Evidence Based Medicine.

Bone Age Retarded			
Addison Disease	Dubowitz syndrome	Juvenil Idiopatic Osteoporosis	Other chronic infections, as Malaria
Amonopterin fetopathy	Elite sports	Juvenil Rhemtaoid Arthritis	Papillon-Lefevre Syndrome
Anemias	Endemic Diarrhea	KBG syndrome	Parasitosis
Aspartylglucosaminuria	Extreme weight loss	Kocher-Debre-Semelaigne Syndrome	Patterson Syndrome
C Syndrome	Fetal rubella syndrome	Laron Dwarfism	Phenylketonuria
Celiac Disease	Freeman-Seldom syndrome	Larsen Syndrome	Pleonosteosis
Cistis fibrosis	Fucosidosis	Legg, Calvé, Perthes Disease	Prader- Willi Syndrome
Cleidocraneal dysplasia	GAP0 syndrome	Lenz-Manjewski hiperostotic dwarfism	Raquitism
Coffin-Lowry Syndrome	Geophagia-dwarfism hipogonadism syndrome	Lesch-Nyhan syndrome	Renal Failure
Coffin-Siris Syndrome	Glycogenosis tipe I	Malnourishment	Renal tubulra acidosis
Crohn Disease	Hipoadrenalism	Marinesco-Sjögren syndrome	Rickets
Cupper Deficiency	Hipogonadism	Mauriac syndrome	Rubinstein-Taybi syndrome
De Lange Syndrome	Hipoparatiroidism	Melnick-Needles osteodysplasty	Silver-Russel syndrome
De Morsier syndrome	Hipopituitarism	Metartropic dysplasia	Talasemia
De Sanctis-Cacchione syndrome	Hipotiroidism	Meyer dysplasia of femoral head	Thalassemia
Deaf mutism-goiter euthyroidism syndrome	Histiocitosis X	Mucolipidosis II	The 3 M syndrome
Deprivation dwarfism	HIV infection	Mucopolysacharidosis	Trichohesis nodosa syndrome
Deprivation Syndrome	Hypogonadysm	Nephrotic syndrome	Trisomy 21
Diabetes Mellitus	Incontinentia pigmenti	Nervous Anorexia	Weill-Marchesani Syndrome
Diabetes Mellitus	Intrauterine growth retardiation	Noonan syndrome	Wilson disease
Drugs	Johanson-Blizzard syndrome	Osteoporosis idiopatic juvenile	Zellweger Syndrome

Table 2. Pathologic factors associated with bone age process retardation.

Case reports like the one published by Nambiar et al in 1998 warns forensic physicians of the real ethical dangers of not correctly expressing the degree of certainty of the results included in their expert reports (Nambiar et al., 1998).



## 8. Expert report

According to the recommendations of the Study Group on Forensic Age Diagnostics (Schmeling et al., 2008) the collected findings and the determined stages are to be presented in detail in the expert report. The used stage classifications and reference studies are to be mentioned.

Reference studies used for forensic age estimation should meet the following requirements:

- Adequate sample size
- Proven age of subjects
- Even age distributions of subjects
- Analysis separately for both sexes
- Information on the time of examination
- Clear definition of the examined features
- Detailed description of the methods
- Data on the reference population regarding ethnicity, socioeconomic status, state of health
- Data on the sample size, mean value, and range of scatter for each examined feature

Examples of reference studies are Greulich and Pyle (Greulich & Pyle, 1959), Gunst et al. (Gunst et al., 2003), Kahl and Schwarze (Kahl & Schwarze, 1988), Kellinghaus et al. (Kellinghaus et al., 2010a, b), Mincer et al. (Mincer et al., 1993), Olze et al. (Olze et al., 2003, 2004b, 2006), Ruhstaller (Ruhstaller, 2006), Schmeling et al. (Schmeling et al., 2004), Tanner et al. (Tanner et al., 2001), Thiemann et al. (Thiemann et al., 2006).

For each examined feature, the report must indicate the most likely age and the range of scatter of the reference population. While mean values and medians show the most likely age for a certain age characteristic standard deviations and interquartile differences are common measurement data for the ranges of scatter. Mean values and standard deviations are valid only for normally distributed features. 68% of the test persons of the reference population with a certain feature lie between the mean value plus/ minus one standard deviation and 95% of the test persons lie between the mean value plus/ minus two standard deviations. Medians and percentiles are distribution independent parameters. 50% of the test persons with a special feature lie within the 25<sup>th</sup> and the 75<sup>th</sup> percentiles. This difference is also called interquartile difference.

It has to be pointed out that means and medians can not be used for the last stage of the age characteristics because they depend on the upper limit of the examined age group. Instead of mean values the 50% probability value should be used for the last stage of an age characteristic. This value can be calculated by means of logistic regression (Knell et al., 2009).

The results of the individual examinations should be compiled in a final age diagnosis. The individual's most likely age is estimated on the basis of all partial diagnoses and a critical discussion of the individual case. This final age diagnosis should include a discussion of the age-relevant variations resulting from application of the reference studies in an individual case, such as different ethnicity, different socioeconomic status or diseases that may affect the development of the individual examined.

However, for age diagnoses obtained with a combination of methods there is still no satisfactory way to scientifically determine the margin of error. While a number of reference studies collected data on individual features and some studies both on skeletal maturation and tooth mineralization (Grön, 1962; Lacey, 1973; Lamons & Gray, 1958; Pfau & Sciulli,

1994), there is still no reference study available analysing all required features for one single reference population. If independent features are examined as part of an age diagnosis that combines several methods, it may be assumed that the margin of error of the combined age diagnosis would be smaller than that for each individual feature. So far it has not been possible to quantify this difference. Since combining methods also makes it possible to identify statistical outliers, the margin of error of the combined age diagnosis should decrease to a certain extent, which unfortunately is also not quantifiable.

Indirect conclusions about the range of scatter of the summarizing age diagnosis were possible after verifying age estimates carried out at the Institute of Legal Medicine in Berlin. To this effect, the court's case files of the persons originally examined for age estimation purposes at the institute were consulted to see whether the actual age of these persons was established during the court proceedings. In all cases where the age of the person concerned could be verified beyond doubt deviation between estimated and actual age ranged between plus or minus 12 months (Schmeling et al., 2003).

Furthermore the expert report should give the degree of probability that the stated age is the actual age or that the individual's age is above the relevant age limit. To this purpose the following probability ratings can be used:

- "almost certain probability (beyond reasonable doubt)": probability > 99.8% (This probability refers to the threefold standard deviation).
- "very probable" or "high probability": probability > 90%
- "probable": probability > 50%
- "undecided": probability = 50%
- "less probable" or "improbable": probability < 50%
- "very improbable": probability < 10%
- "almost certainly improbable": probability < 0.2%

The following phrasing gives an example for an adequate conclusion:

"Summarizing all test results the following can be established:

- There is a very high probability that the given date of birth is not correct but that an earlier date can be assumed,
- there is a very high probability of an age of above 14 years and a high probability of one above 16,
- there is but very low probability that the 18th year of age has been reached,
- and there is almost certain probability that the 21st year of age has not been reached."

## 9. Practical cases

### Case 1

The accused, of Afghan origin, was under investigation on murder charges. According to his own statement he was 13 years and 5 months old at the time of examination. As the investigative authorities had considerable doubts relative to the age given by the person concerned, a court order was issued for forensic age estimation with inclusion of X-ray examinations.

In the course of physical examination, a body length of 168 cm was measured, body weight was 55 kg. The upper lip and chin were covered with 5 mm-long beard growth, the cheeks manifested an after-shave condition. The larynx was prominent. The armpits displayed 5

mm-long hair stubble. The genital region manifested a dense area of curly hair rising up sparsely to the navel. Genital development was to be placed in the second phase of puberty. The physical stature and the pattern of body hair corresponded to those of an adolescent. No indications of developmental disorders were detected.

Fig. 5 shows the hand radiograph of the person concerned. The X-ray morphology of the carpal bones appeared normal. The former epiphyseal plates of the metacarpals and phalanges were completely ossified and in part still slightly marked. The distal epiphyseal plates of the radius and ulna were for the most part open and presented incipient ossification only in the middle section. On the basis of the morphological findings of the X-rays, a skeletal age of 16-17 years according to Thiemann et al. (Thiemann et al., 2006) emerged. In the reference study by Schmeling et al. (Schmeling et al., 2006), for a skeletal age of 16 years a mean age of 15.3 years with a standard deviation of 0.8 years was reported. For a skeletal age of 17 years, the mean age is 16.8 years, whilst standard deviation amounts to 1.1 years. A reference study which gathered data from a socioeconomically highly developed population was used. For this reason, it may be assumed that the actual age of the person concerned does not lie below the estimated age. As the development of the hand skeleton was not complete, no X-ray examination of the clavicles was performed.

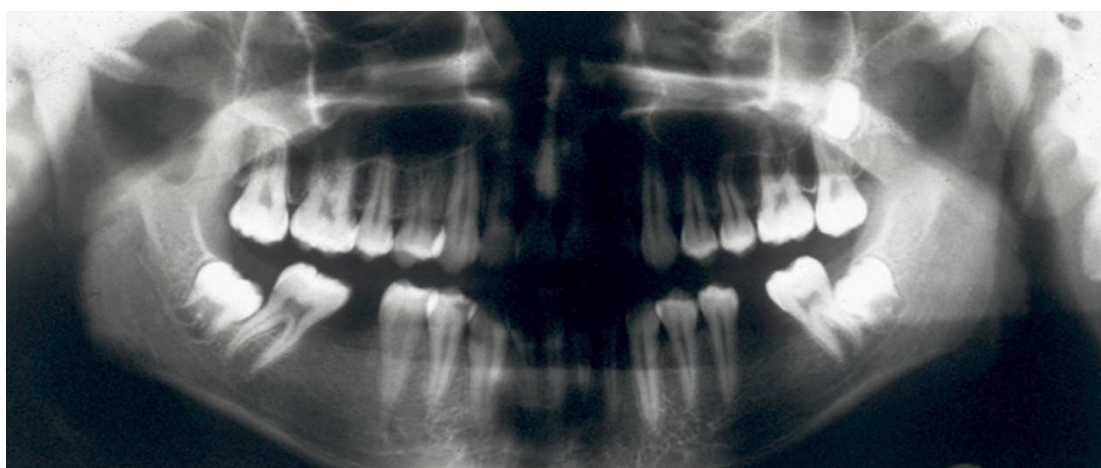


Fig. 5. Case 1: orthopantomogram

In the course of dental examination it was ascertained that the third molars had not erupted into the oral cavity. An evaluation of the orthopantomogram revealed that tooth 18 was congenitally absent and that impacted tooth number 28 presented a mineralisation stage D according to Demirjian whilst teeth 38 and 48 presented a stage E (fig. 6). In the reference study by Olze et al. (Olze et al., 2003), a mean age of 16.3 years was given for mineralisation stage D of tooth number 28 with a standard deviation of 3.2 years. The mean age for stage E of the lower third molars is 16.7 years with standard deviations of 2.1 to 2.3 years. On the basis of the ethnicity of the person concerned, a reference study for Caucasians was used.

In the synopsis of the findings it was determined that the person concerned was most probably 16-17 years old at the time of examination. At the time of examination the 14<sup>th</sup> year of life had been completed beyond reasonable doubt. The age stated by the person concerned was not consistent with the examination findings. In the course of the court case it came to light that the actual age of the person concerned at the time of examination was 16 years and 4 months.



Fig. 6. Case 1: hand radiograph

#### Case 2

The person to be examined was under investigation for a drug offence. According to his own statement the accused came from Guinea-Bissau and was 17 years and 8 months old at the time of examination. As the investigative authorities had considerable doubts relative to the age given by the person concerned, a court order was issued for forensic age estimation with inclusion of X-ray examinations.

In the course of physical examination, a body length of 178 cm and a body weight of 68 kg were measured. The upper lip, chin and cheeks manifested an after-shave condition. The larynx was prominent. The armpits displayed an area of dense curly hair. The genital region was covered with a horizontally restricted area of dense curly hair. The external genitalia were mature. The physical stature and the pattern of body hair corresponded to those of an adult. No indications of developmental disorders were detected.

Fig. 7 shows the hand radiograph of the person concerned. The X-ray morphology of the carpal bones appeared normal. The former epiphyseal plates of the metacarpals and



phalanges were completely ossified and no longer detectable. The former epiphyseal plate of the radius was completely ossified and only very discretely marked in the middle third. The former epiphyseal plate of the ulna was completely ossified and no longer detectable. Ossification of the hand skeleton was thus complete. In line with this, skeletal age according to Thiemann et al. (Thiemann et al., 2006) was 18 years. In the reference study by Schmeling et al. (Schmeling et al., 2006), for a skeletal age of 18 years a mean age of 18.2 years with a standard deviation of 0.7 years was reported. The minimum age was 16.7 years. A reference study which gathered data from a socioeconomically highly developed population was used. For this reason, it may be assumed that the person concerned was not younger than 16.7 years at the time of examination.



Fig. 7. Case 2: hand radiograph

As development of the hand skeleton was complete, a CT examination of both sternoclavicular joints was performed with a slice thickness of 1 mm. The former medial clavicular epiphyseal plate was completely ossified on both sides. In the region of the former epiphyseal plates, remnants of the epiphyseal scar still remained both on the right and the left (fig. 8 and 9). Thus, a stage 4 was present on both sides according to Schmeling et al. (Schmeling et al., 2004). In the reference study by Kellinghaus et al. (Kellinghaus et al., 2010) the mean age for stage 4 was 29.6 years with a standard deviation of 4.2 years. The minimum age was around 21.6 years.

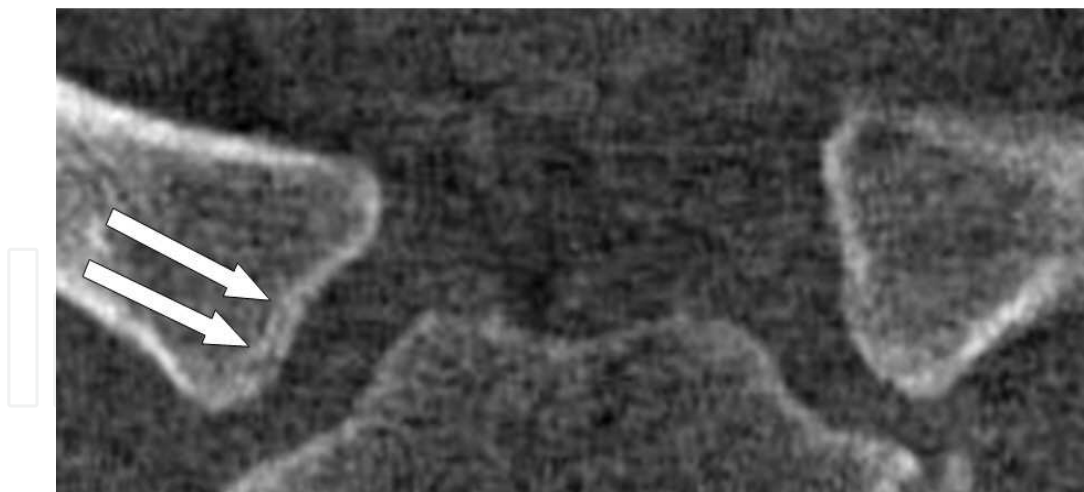


Fig. 8. Case 2: CT scan of the sterno-clavicular joints, the arrows show remnants of the epiphyseal scar of the right clavicle

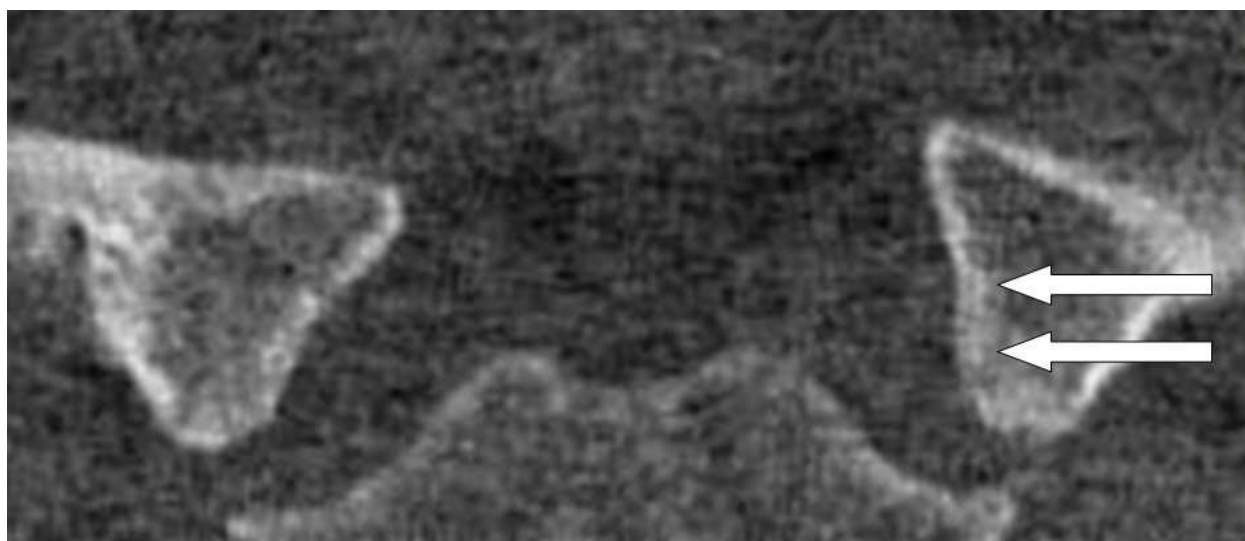


Fig. 9. Case 2: CT scan of the sterno-clavicular joints, the arrows show remnants of the epiphyseal scar of the left clavicle

In the course of dental examination it was ascertained that the person concerned had an incomplete set of teeth. Teeth numbers 11, 12, 21, 22 and 37 were missing. All four third molars had erupted into the oral cavity and had reached the occlusal plane. An evaluation of the orthopantomogram revealed that all the third molars presented a mineralisation stage H according to Demirjian (fig. 6). In the reference study by Olze et al. (Olze et al., 2006) a mean age of between 22.7 and 22.9 years was given for mineralisation stage H of the third molars with standard deviations of between 2.3 and 2.5 years. The minimum age was given as 17 years (Olze et al., 2004). On the basis of the ethnicity of the person concerned a reference study for black Africans was used.

In the synopsis of the findings it was determined that the absolute minimum age of the person concerned was 21.6 years. At the time of examination both the 18th and the 21st year of life had been completed beyond reasonable doubt. The age stated by the person concerned was not consistent with the examination findings.



Fig. 10. Case 2: orthopantomogram

## 10. Reference

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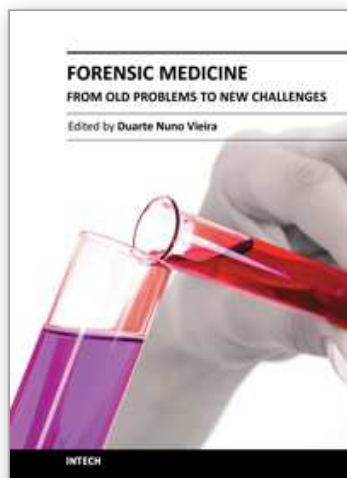
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