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ORIGINAL ARTICLE

Accuracy of scoring of the epiphyses at the knee joint (SKJ) for assessing legal adult age of 18 years

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Abstract Important aspects of forensic practice are age estimation and discrimination of individuals of unknown age as adults and minors. The developing knee joint was recognized as a potential site for age examination in late adolescence. We analyzed a sample of anteroposterior x-rays of the knee joints from 446 living individuals from Umbria, Italy (234 males and 212 females), aged between 12 and 26 years. We evaluated the ossification of the distal femoral (DF), proximal tibial (PT), and proximal fibular (PF) epiphyses. We took into account possible persistence of the epiphyseal scars in the ossified epiphyses by the adopted stages of those previously introduced by Cameriere et al. (2012). We also used measurements from all three epiphyses to calculate the total score of maturation for the knee joint (SKJ). Cohen Kappa coefficients of intrarater agreement for staging the DF, PT, and PF epiphyses were 0.839, 0.894, and 0.907, while interrater agreement was 0.919, 0.791, and 0.907, respectively. The resulting receiver operating characteristic (ROC) curves of SKJ show better discriminatory power than those for DF, PT, and PF epiphyses in predicting that the participant, either male or female, was an adult or a minor. The areas under the curves for SKJ were 0.991 and 0.968 vs. 0.944, 0.962, 0.974 and 0.891, 0.910, 0.918 for males and females, respectively. The results of the 2 by 2 contingency tables showed that SKJ score of 4 in males and SKJ score of 5 in females were the most suitable cut-off value in discriminating between adults and minors. Principally, the sensitivity test for males was 0.94, with 95 % confidence interval (95 % CI) 0.90 to 0.97 and specificity was 0.96 (95 % CI 0.91 to 0.98). The proportion of correctly classified individuals was 0.95 (95 % CI 0.91 to 0.97). For females, the sensitivity test was 0.89 (95 % CI 0.84 to 0.92) and specificity was 0.92 (95 % CI 0.87 to 0.96), the proportion of correctly classified individuals was 0.90 (95 % CI 0.85 to 0.94). These results indicate that the SKJ method may give valuable supporting information in forensic procedures for discriminating individuals of legal adult age of 18 years. Further studies should address the usefulness of the SKJ method in different populations.

Keywords Forensic science · Knee joint · Ossifying epiphyses · Unaccompanied minor · Age estimation · Adult age

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Introduction

In the past decades, forensic anthropology has become an integral part of forensic science, addressing research areas related to populations and demographic characteristics such as determination of age, sex, stature, and race for different purposes [1]. Forensic anthropology also studies the skeletal

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remains as part of a judicial investigation in order to identify the circumstances and causes of the death and provides useful information for identifying a corpse [1].

Verified personal documentation and birth certificates are the only way to know the exact age of an individual. However, for individuals who do not possess such documents, it is of highest importance to verify whether these persons will be treated as juveniles or adults by governmental administrations and authorities. This is important not only in legal and criminal prosecutions but also in civil hearings, including determination of refugee status [2–6]. Although age thresholds vary in different countries, relevant age for criminal liability ranges between 14 and 18 years [7–9].

In Italy, a person's age determines the accessibility of services such as child protection, education, and healthcare during childhood, as well as different benefits, citizen rights during adulthood, including employment legislation, banking services, drivers' licenses, and pension eligibility [10, 11]. Full legal responsibility is acquired at 18 years of age [12].

To estimate the age of an individual for whom proper documentation is not available, forensic experts must use an ethical and scientific approach which relies on validated population data concerning growth and development [13–16]. This approach enables creation of a biological profile of the developmental status of an individual of unknown age, which is then the basis for estimating age. This profile is based on growth markers of specific anatomical structures, mostly skeletal (hand and wrist, long bones, vertebrae, clavicle) and dental features [17–23]. The possibility of age estimation using the measures of the bones and teeth is an important part of forensic anthropology and can help in providing evidence in cases of illegal migration [1, 24].

The Study Group on Forensic Age Diagnostics (AGFAD) created and updated the guidelines for age estimation, which include a consensus among scientists about the most appropriate methods to use in specific situations, drawing up recommendations for age estimation and institutionalization of quality control, with special attention to sensitive legal and ethical implications [25]. Cunha et al. [2] suggest that in cases of age estimation, the method used has to be considered applicable and should follow specific requirements, and be presented to the scientific community, as a rule by publication in peer-reviewed journals. Clear information concerning the accuracy of age estimation using the method should be available, and in cases of age estimation in living individuals, the principles of medical ethics and legal regulations have to be considered [2]. According to AGFAD, skeletal maturation is evaluated from the left hand radiographs; if ossification is completed, an additional examination of clavicles is required [26]. The impact of race and socioeconomic, pathological, geographical, and temporal variability on skeletal development of the left hand was described in detail by Garamendi et al. [24].

Special attention should be given to possible risks from radiologic examination, with recommendations that all unnecessary or overdosed exposure to x-rays must be avoided. There is vigorous debate in recent literature about various ethical aspects, differences of age threshold in many countries, and levels of probability requested by legal rules for age estimation in criminal and civil cases [11, 27, 28]. Recent studies have made significant breakthrough in the application of non-invasive imaging procedures in estimating the age of living subjects, predominantly magnetic resonance imaging (MRI) and ultrasound examination [29–40].

The knee is the anatomical structure that can provide a great amount of data for research on age estimation. The articulation surfaces of three different bones, the distal femur (DF), the proximal tibia (PT), and the proximal fibula (PF), build the knee joint. It is an anatomical area, which is easy to radiograph at low radiation doses, easily positioned for anteroposterior x-rays, and with no interposed anatomical structures [41–43].

There are numerous other anthropological studies of skeletal maturation of the knee based on dry bone, x-ray, and MRI, but they differ with regard to numerous variables: study populations, gender, number of individuals, age range, and number of bone fusion stages [32, 33, 36, 37, 43–48]. The Pyle and Hoerr [42] atlas of skeletal development of the knee from 1955 has a main application in estimating the developmental level of children's knees from x-ray films. The atlas was based on a longitudinal film series study from birth until the age of 18 years in healthy white individuals from Cleveland. The participants consisted of males and females, known age and favorable social backgrounds, with the aim to studying different influences, such as nutrition or diseases, on growth and development. Roche et al. [49] introduced a method of skeletal age estimation using only a frontal view of the knee (RWT). Contrary to the Pyle and Hoerr's [42] atlas, RWT provides the normal ranges of skeletal age and the error of the estimate. RWT used a total of 16 tibial, 12 femoral, and 6 fibular indicators and a computer program for the evaluation of skeletal maturation. The most comprehensive and accurate anthropological study of the knee was that of McKern and Stewart, who examined the bodies of young North American soldiers killed during the Korean War [50]. They classified the processes of epiphyseal union at the knee into five stages; from non-union to complete union. O'Connor et al. [43, 45] revisited these stages in a contemporary sample of Irish individuals aged 9 to 19, provided detailed description and x-rays specific for each of the stages of union, and studied morphological changes of the epiphyses at the knee joint using 7 (A–G) modified RWT criteria. Additionally, O'Connor et al. [44] created specific regression formulae to bone age calculation from the combination of morphology changes of the epiphyses, also adding three (H–J) stages. Morphology and epiphyseal changes occurred earlier in females in the Irish population, which corresponds with previous studies of the knee [43]. Population specific profiles of age range of a specific maturation process have been well documented by Schaefer and Black's [47] comparative study on skeletal remains of the ages on the epiphyses between young Bosnian males from Srebrenica and North American males from the Korean War. Each of the ten epiphyses in their study reached complete fusion in the Bosnian male sample, 1 to 3 years earlier than in the North Americans [47].

Cameriere et al. [51] in 2012 studied the frontal radiographs of epiphyseal fusion at the knee joint in Italian participants aged between 14 and 24 years to assess likelihood of having attained 18 years of age. In their study, they differentiated three stages of final maturation of DF, PT, and PF epiphyses: stage 1, the epiphysis is not fused; stage 2, epiphysis is fully ossified and epiphyseal scar is visible; and the final, stage 3, epiphysis is fully ossified and epiphyseal scar is not visible. Scores of 0, 1, and 2 were assigned to the abovementioned stages 1, 2, and 3. Lastly, the total score, related to the epiphyseal fusion at the knee joint (SKJ), calculates the sum of all three scores of the DF, PT, and PF. The highest value of accuracy (Acc) in discrimination between adults and minors was obtained with SKJ score of 3 (Acc = 91.38 %) for boys and SKJ score of 4 (Acc = 85.86 %) for girls.

Recent studies showed that epiphyseal scars may be persistent for decades after fusion and should not be used as evidence of recent bone fusion for age estimation purposes [52–54]. Faisant et al. [54] also showed that, if the epiphyses of the knee were classified according to a visible scar or no scar, all individuals without a scar were at least 18 years of age. These studies indicated that the analysis of radiographs of the knee can provide useful information on the age of individuals and contribute to the decision on the attainment of the age of majority. We decided to evaluate stages of the epiphyseal fusion at the knee joint and their usefulness in relation to legal adult age of 18 years in a decisive manner on a new sample of living participants, taking into consideration expected presence of the scar in individuals.

Materials and methods

This was a cross-sectional, retrospective study based on the sample of the frontal radiographs of the left knee with anteroposterior incidence, carried out between 2013 until the end of 2015 at Foligno Hospital in the region of Umbria, Italy. A total of 446 radiographs of Caucasian Italian participants (234 males and 212 females), aged between 12 and 26 years, were analyzed. Indications for x-ray examination were injuries and evaluation of fractures at the knee region, pain and swelling of the knee joint, osteochondritis dissecans, and orthopedic follow-up. Table 1 lists the age and gender distribution for each age category.

We excluded radiographs showing fractures or dislocations involving the growth plate or those that showed surgical implants or fixatives near the diaphyseal–epiphyseal junction. We also excluded radiographs obtained from subjects with a medical history of chronic disease or with known endocrine, metabolic, or nutritional disorders which may significantly alter skeletal development. Socioeconomic and competitive sports activity statuses of participants were not recorded.

All radiographs were obtained with the same device settings and technical data: Focus Film Distance 110 cm, no grid, 110 mA, 7 mAs, 55 kV, cassette size 24 × 30 cm; the server used to store and analyze digital images is an AGFA, PACS model. The chronological age for each participant was calculated as the difference between the dates of birth and the x-ray.

The DF, PT, and PF epiphyses were separately evaluated for the degree of the ossification according to three different stages as follows: stage 1, if epiphysis is not fused (Figs. 1a, 2a, and 3a); stage 2, epiphysis is fused, and epiphyseal scar is clearly visible, fully spreading on the whole length in a mediolateral direction, where lateral sides may not be completely ossified (Figs. 1b, 2b, and 3b); and stage 3, epiphysis is fully ossified and the traces of epiphyseal scar may be visible (Figs. 1c, 2c, and 3c). The stages of the ossification of DF, PT, and PF epiphyses, 1, 2, and 3, were assigned to scores 0, 1, and 2, respectively. Subsequently, the sum of all three epiphyseal scores represents their total score (0 to 6), or SKJ. Particular attention is necessary when the epiphysis of the femur or tibia shows complete ossification, but the observed lateral sides are not completely ossified [51]. In this case, the maturation stage is assigned as score 1. In cases of uncertainty during observation, the lower stages were assigned, according to the principle of benefit of the doubt, used in criminal proceedings [51].

Statistical analysis and data management

MS Excel 2003 (Microsoft Office 2003, Microsoft, Redmond, WA) and IBM SPSS Statistics 17.0 for Windows (SPSS Inc., Chicago, IL) were used for all data management and statistical analysis. All evaluation of the DF, PT, and PF epiphyses stages and calculation of the SKJ were done by the same author (AG). Cohen Kappa was used for intrarater and interrater agreement of the DF, PT, PF, and SKJ epiphyses stages of maturation. Fifty randomly selected x-rays were scored 2 months later by the same author (AG) for intrarater agreement and the second author (FM) for interrater agreement.

Table 1 Frequency distribution by sex and age cohorts

Sex	Age														
	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Total
Males	8	14	21	23	21	23	19	18	14	19	11	27	5	7	4	234
Females	5	13	25	25	14	23	21	22	12	9	10	5	12	7	9	212
Total	13	27	46	48	35	46	40	40	26	28	21	32	17	14	13	446

The predictive accuracy of the specific epiphysis and the SKJ, to test whether a participant is 18 years and older (adult) or under 18 years (minor), was assessed by determining the receiver operating characteristic (ROC) curve. The area under the curve (AUC) and 95 % confidence interval (95 % CI) and standard error (SE) of AUC was calculated with a non-parametric method to evaluate the discriminatory ability or accuracy of the specific epiphysis and the SKJ in males and females [51, 55, 56]. Briefly, the closer to the apex of the ROC curve to the upper left corner, the greater the accuracy of the test [24, 51].

Different values of SKJ score for males and females were tested to consider that an individual was ≥18 years or <18 years, and the percentage of accurately classified participants were evaluated, as recommended by Cameriere et al. [51]. For each SKJ score in males and females separately, contingency 2-by-2 table lists the numbers of individuals whom specific SKJ score select those who are 18 years and older (True positive, TP), those who are younger than 18 years (False positive, FP), those with specific SKJ score who are 18 years and older (False negative, FN), and finally those with a specific SKJ score who are younger than 18 years (True negative, TN) [57]. The sensitivity of the test (p_1), (i.e., the proportion of subjects older than 18 years of age who have SKJ ≥specific score) were evaluated, together with specificity (p_2) (i.e., the proportion of individuals younger than 18 who have SKJ <specific score). The positive predictive value (PPV) of the test is the probability of being an adult in participants with positive results, i.e., those with a specific SKJ score or more, while the negative predictive value (NPV) is the probability of being a minor if SKJ score is under a specific cut-off [57]. Likelihood ratios are an additional way of describing the performance of the discrimination of specific cut-off values or how many times more or less likely a result is to be found in adults or minors. Results are presented in odds ratios. In this study, the positive likelihood ratio (LR+) is the ratio of the proportion of TP with (TP + FN) to the proportion of FP with a FP + TN. The negative likelihood ratio (LR−) is the ratio of the proportion of FN with (TP + FN) to the proportion of TN with FP + TN [57]. Higher values of LR+ and lower values of LR− better refer to the ability of a particular cut-off value in identifying participants properly [57, 58]. The Bayes posttest probability (Bayes PTP) of being 18 years of age or more of the SKJ was also evaluated [51]. According to Bayes' theorem, posttest probability can be calculated as

$$p = \frac{p_1 p_0}{p_1 p_0 + (1-p_2)(1-p_0)} \quad (1)$$

where p is posttest probability and p_0 is the probability that the subject in question is 18 or older, given that the individual is aged between 12 and 26 years, which represent the target population. Probability p_0 was evaluated with data from the Italian National Institute of Statistics for 2014, ISTAT [59] and was considered to be 0.62 for males and females.

Results

Mean age of tested sample was 18.65 ± 3.65 and 18.50 ± 3.72 years in males and females, respectively without a statistically significant difference ($p = 0.660$). Spearman correlations between the scores of maturation of the DF, PT, and PF epiphyses and real age were 0.835, 0.872, and 0.878 and

Fig. 1 Distal femoral epiphysis: **a** Stage 1, epiphysis is not fused; **b** Stage 2, epiphysis is fused, and epiphyseal scar is clearly visible, fully spreading on the whole length in a mediolateral direction, where lateral sides may not be completely ossified; **c** Stage 3, epiphysis is fully ossified and the traces of epiphyseal scar may be visible

Fig. 2 Proximal tibial epiphysis: **a** Stage 1, epiphysis is not fused; **b** Stage 2, epiphysis is fused, and epiphyseal scar is clearly visible, fully spreading on the whole length in a mediolateral direction, where lateral sides may not be completely ossified; **c** Stage 3, epiphysis is fully ossified and the traces of epiphyseal scar may be visible

0.789, 0.799, and 0.826 for males and females, respectively, while the correlation between SKJ and real age were 0.899 in males and 0.881 in females ($p < 0.01$).

The Cohen Kappa coefficients for intrarater agreement were 0.839, 0.894, and 0.907 and 0.919, 0.791, and 0.907 for interrater agreement for the DF, PT, and PF epiphyses stages of maturation, respectively.

Table 2 shows the values of the chronological age (years) at each stage of ossification for each of the three epiphyses at the knee, for males and females separately. The mean ages between sexes varied across the stages of maturation of all three epiphyses without statistically significant difference. Both the real age and the SKJ gradually increased for both sexes (Fig. 4). The mean ages between sexes varied across the SKJ scores and the greatest difference was found in the SKJ score 4 (18.32 ± 1.02 and 17.67 ± 1.42 years in males and females, respectively) without a statistically significant difference ($p > 0.05$) (Table 3).

The resulting ROC curves of SKJ scores show better discriminatory power than the DF, PT, and PF epiphyses scores in males and females (Fig. 5). The values for AUC were 0.991 and 0.968 for the SKJ vs. 0.944, 0.962, and 0.974 and 0.891, 0.910, and 0.918 for the DF, PT, and PF for males and females, respectively (Table 4).

The efficiency and validity of cut-off values of different SKJ scores for discriminating participants between adults and minors were tested, and the values of the test were presented separately for males (Table 5) and females (Table 6). Generally, close association was found between adult age and the results of SKJ ≥4 in males (Table 5) and SKJ ≥5 in females (Table 6).

In males, 222 out of 234 participants were accurately classified as adult or minor for SKJ ≥4, Acc = 0.95 (95 % CI 0.91 to 0.97). The results show that the sensitivity p_1 of the measure for males was 0.94 (95 % CI 0.90 to 0.97) and specificity p_2 was 0.96 (95 % CI 0.91 to 0.98). PPV of the test was 0.96 (95 % CI 0.92 to 0.98). LR+ and LR− were 20.76 (95 % CI 10.13 to 48.10) and 0.06 (95 % CI 0.03 to 0.10), respectively. The estimated Bayes posttest probability p was 0.97 (95 % CI 0.91 to 1.00). In females, 192 out of 212 subjects were accurately classified for SKJ ≥5, Acc = 0.90 (95 % CI 0.85 to 0.94). These results show that p_1, or the proportion of individuals being 18 years of age or older whose test was positive, was 0.89 (95 % CI 0.84 to 0.92) and specificity p_2, the proportion of individuals younger than 18 years whose test was negative, was 0.92 (95 % CI 0.87 to 0.96). PPV of the test was 0.92 (95 % CI 0.87 to 0.96). LR+ and LR− were 11.65 (95 % CI 6.50 to 22.65) and 0.12 (95 % CI 0.08 to 0.19) respectively. The estimated Bayes posttest probability p was 0.95 (95 % CI 0.88 to 1.00).

Discussion

The anteroposterior x-rays of the knee were recognized as a potential useful marker for discriminating individuals as older than 18 years and minors [51, 54]. The traces of an epiphyseal scar was taken into consideration in this study and the stages of bone fusion, proposed by Cameriere et al. [51], were changed with the new classification, because previous studies confirmed persistence of this phenomenon, mostly affecting lower limbs [54, 60]. This is the first study that validated the different stages and new values of SKJ for males and females in a new sample of participants from Italy.

Comparison of the individual epiphyses and SKJ scores as estimates that participants are adults or minors, by the

Fig. 3 Proximal fibular epiphysis: **a** Stage 1, epiphysis is not fused; **b** Stage 2, epiphysis is fused, and epiphyseal scar is clearly visible, fully spreading on the whole length in a mediolateral direction, where lateral sides may not be completely ossified; **c** Stage 3, epiphysis is fully ossified and the traces of epiphyseal scar may be visible

Table 2 Statistics of chronological age across scores of maturation (Score) 0, 1, and 2 of the distal femoral (DF), proximal tibial (PT), and proximal fibular (PF) epiphyses

Epiphysis	Score	Males	Age[a]								Females	Age[a]								P^b
		N	Mean	Mean − 2SD	Mean + 2SD	Min	Q_1	Med	Q_3	Max	*N*	Mean	Mean − 2SD	Mean + 2SD	Min	Q_1	Med	Q_3	Max	
DF	0	64	14.62	11.84	17.40	12.12	13.75	14.31	15.31	17.68	46	14.56	11.70	17.42	12.02	13.59	14.37	15.47	17.85	0.836
	1	57	17.47	15.24	19.70	14.20	16.05	16.91	18.42	23.45	61	16.84	12.98	20.70	14.16	15.31	16.23	18.46	22.78	0.106
	2	113	21.53	16.85	26.21	16.67	19.44	21.49	23.37	26.79	105	21.18	15.22	27.14	15.32	18.50	20.75	24.06	26.93	0.354
PT	0	70	14.67	12.00	17.34	12.12	13.87	14.34	15.45	17.81	64	14.78	12.09	17.47	12.02	13.76	14.65	15.65	17.66	0.632
	1	62	17.73	14.12	21.34	14.48	16.49	17.43	18.71	23.10	54	17.94	13.14	22.74	14.35	16.19	17.30	19.46	24.10	0.590
	2	102	21.94	17.59	26.29	17.29	20.14	22.06	23.46	26.79	94	21.34	15.50	27.18	15.13	18.85	20.68	24.10	26.93	0.109
PF	0	69	14.64	12.11	17.17	12.12	13.86	14.39	15.43	17.50	46	14.31	12.02	16.60	12.02	13.59	14.28	15.20	17.44	0.168
	1	58	17.50	14.05	20.95	14.30	16.43	17.31	18.15	22.50	76	17.34	13.11	21.57	14.16	15.78	17.14	18.24	24.50	0.643
	2	107	21.86	17.53	26.19	18.00	19.87	21.98	23.45	26.78	90	21.61	16.00	27.22	15.67	19.26	21.33	24.10	26.93	0.495

N number of individuals, *2SD* two standard deviations, *Min* minimum, Q_1 first quartile, *Med* median, Q_3 third quartile, *Max* maximum

[a] Data of age are based on the observed cohort and not the whole population

[b] Independent samples *t* test between sexes

evaluation of a ROC curve and AUC, confirmed an advantage of screening of the complete joint at the knee around a cut-off age of 18 years [51]. Values of AUC including the sensitivity and the specificity of the test would be reduced if the epiphyses were used separately so any single epiphysis would be less effective in its purpose. The sample was balanced for male and female age and we found no significant difference between sexes in stages of ossification of all three epiphyses and SKJ, which is in line with findings by Cameriere et al. [51]. Kappa results showed very good agreements for the same observer and between two different observers, especially if we take into account the possibility of wrong identification, of the residual scar or stage 3 as stage 2 or vice versa.

The present study has main outcomes in comparison to the study by Cameriere et al. [51]. The results of our study showed the best performance of the cut-off value of SKJ score of 4 in males and SKJ score of 5 in females, with correct discrimination between adults and minors of 95 % males and 90 % females, respectively. These findings are different than suggested values of SKJ score of 3 in males and SKJ score of 4 in females which was established on a previous approach and sample also from Italy [51]. The differences could be attributed to the different staging in our study, which recognizes evidence of scars in the final stage, as well as a greater sample size and better distribution across the age range, and possible socioeconomic difference in the ethnical and geographical surroundings similar to the previous study.

Males showed all the best values of the discrimination tests for the suggested score of 4 including values of positive and negative likelihood ratios. Lowering the cut-off value to 3 would reduce the specificity of the test, which is ethically unacceptable according to Garamendi et al. [24]. Females showed the best of the test values of the discrimination tests for the cut-off value 5 of SKJ, including almost the best accuracy and the best specificity which is mandatory for forensic purposes [24]. Only the cut-off value 4 of SKJ shows comparable values for discriminating between adults and minors, including lower specificity and better sensitivity.

Mean findings of age are based on the observed cohort and not on the whole population. Precisely, while the intermediate stage is more independent and appropriate for comparison, the chronological age of the first and last stages is limited and biased by the lowest and the highest age of the participants. The mean age of the intermediate stage, at all three epiphyses, was without significant difference between sexes. Only DF epiphysis in females showed considerably earlier maturation when compared to males but without statistical significance, which indicates the contribution of early maturation of the DF epiphysis in females to the overall lower mean ages of SKJ scores, except in the last score. It is also evident that the mean age in females within the SKJ score of 5 is under the cut-off value of 18 years, which indicates some degree of earlier maturation of the knee in females in the tested sample. Evidence

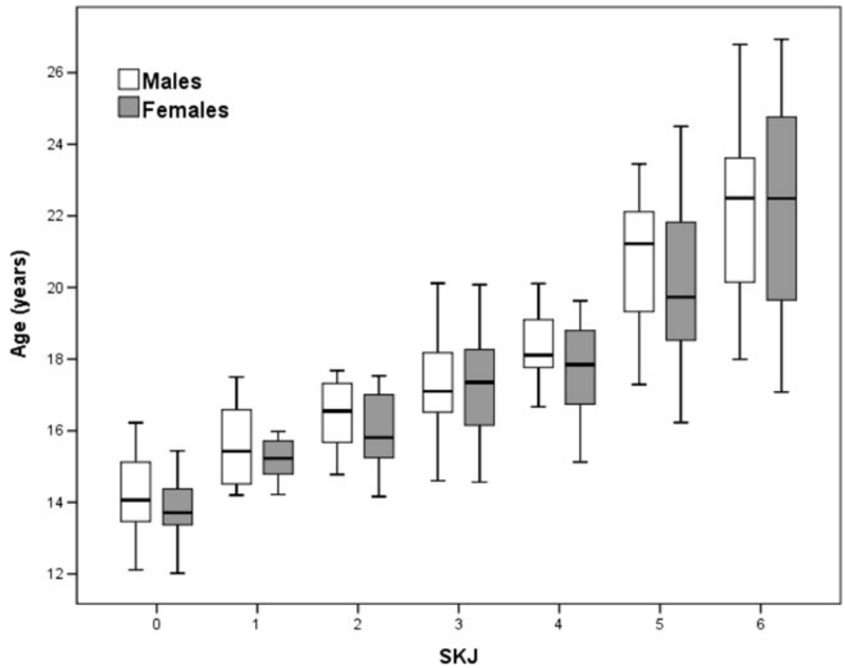


Fig. 4 Boxplot of relationship between chronological age and total score of the epiphyseal fusion at the knee joint (*SKJ*). *Boxplot* shows median and interquartile ranges, whiskers are highest and lowest values

of higher scores of SKJ, 5 in males and 6 in females, indicate full ossification of the knee and higher likelihood of being an adult.

The difference in SKJ score between sexes is principally in line with the study by Cameriere et al. [51], which showed the highest value of predictive accuracy of discrimination between adult and minor for their SKJ scores of 3 and 4 for males and females, respectively. In their study, the ROC curves and values of the 2-by-2 tables showed better specificity for girls for their SKJ score of 4 than obtained with their SKJ score of 3 [51].

The results from our study indicate the potential of the knee and presented approach for scoring of all three epiphyses on anteroposterior x-rays in the SKJ methods to provide important information in cases where discriminating the individuals between adults and minors is necessary. From an expert's point of view, when a report has to be written with the degree of probability that a subject reached legal adult age, the results obtained by the SKJ method could be rated as "very probable" or "highly probable" because of the over 90 % probability [61].

Although the development of the knee can be traced from birth, research has mainly been focused on the completion of ossification. A summary of studies providing age ranges for the completion of epiphyseal union at the knee was presented elsewhere by O'Connor et al. [43]. O'Connor et al. [43] noted that epiphyseal union in females occurred earlier than in males, with significant difference between the mean age of union between sexes for each of stages 1 and 2 for the femur and stages 0, 1, 2, and 3 for the tibia and the fibula. Epiphyseal union at the knee attained completion between 16 and 19 years for males and 14 to 19 years in females in the Irish population, which is in line with age ranges from previous studies [43]. Another radiographic study on Scottish males and females from birth to 20 years reported that no maturational changes were visible at the knee after 19 years in males and 16 years in females [46]. The abovementioned studies on maturation of the epiphyseal union at the knee are hard to compare with our study because of different gender and sample distribution and different indicators of maturation.

Our study was a cross-sectional study of x-rays of participants of a certain age range, which were radiographed for different clinical reasons and represent a contemporary Italian population, of similar or of the same ethnicity to participants in the study by Cameriere et al. [51]. They live in a specific geographic region, different to the pilot study. Generally, it is considered that ethnicity does not affect the skeletal development while socioeconomic status does [62, 63]. Yet, data on socioeconomic status was not recorded in this study, which with certain pathological factors and activity in competitive sports of a participant may affect skeletal development [24]. So, the accuracy of this method should be applied judicially and carefully in each case and should be compared with other developmental indicators, especially if evaluated in different populations than the original one, because epiphyseal ossification of the knee could be socioeconomically dependent and population-specific [43, 47, 63, 64]. The AGFAD criteria for age estimation in living individuals takes into account not just the indicator of age of the specific anatomic region but also a detailed physical examination which should include anthropometric measures, signs of sexual maturation, and a dental examination [11, 26, 65]. Dental

Table 3 Statistics of chronological age across scores of the epiphyseal fusion at the knee joint (SKJ)

SKJ score	Males									Females									
		Age[a]									Age[a]								
	N	Mean	Mean − 2SD	Mean + 2SD	Min	Q_1	Median	Q_3	Max	*N*	Mean	Mean − 2SD	Mean + 2SD	Min	Q_1	Median	Q_3	Max	P^b
0	49	14.12	12.10	16.14	12.12	13.40	14.06	15.16	16.22	31	13.77	12.05	15.49	12.02	13.34	13.71	14.45	15.44	0.192
1	16	15.61	13.38	17.84	14.20	14.50	15.43	16.62	17.50	12	15.21	14.09	16.33	14.22	14.74	15.22	15.74	15.98	0.980
2	23	16.47	14.65	18.29	14.78	15.55	16.55	17.34	17.68	35	15.97	13.91	18.03	14.16	15.20	15.81	17.03	17.53	0.056
3	24	17.33	14.92	19.74	14.60	16.50	17.10	18.20	20.12	15	17.30	14.30	20.30	15.56	16.07	17.35	18.68	20.08	0.306
4	9	18.32	16.32	20.32	16.67	17.68	18.11	19.16	20.11	16	17.67	14.89	20.45	15.13	16.67	17.85	18.82	19.63	0.609
5	27	20.77	17.40	24.14	17.29	19.21	21.22	22.12	23.45	40	20.18	15.83	24.53	16.23	18.46	19.73	21.97	24.50	0.772
6	86	22.12	17.69	26.55	18.00	20.14	22.49	23.64	26.79	63	22.27	16.63	27.91	17.08	19.63	22.49	25.02	26.93	0.436

N number of individuals, *2SD* 2 standard deviations, *Min* minimum, Q_1 first quartile, *Med* median, Q_3 third quartile, *Max* maximum

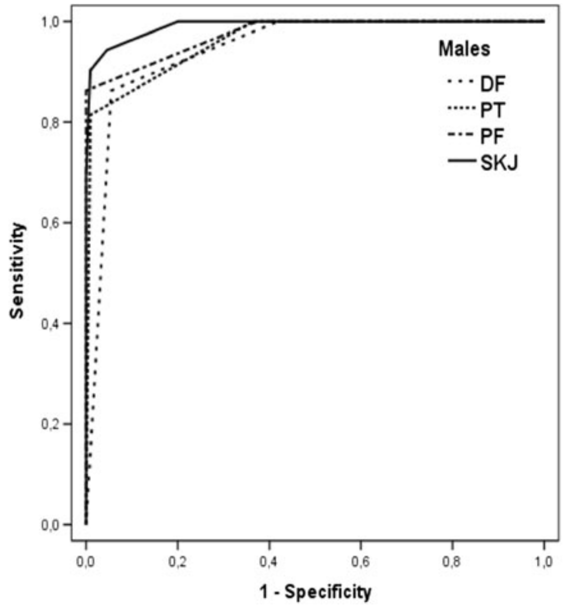
[a] Data of age are based on the observed cohort and not the whole population

[b] Independent samples Mann–Whitney test

examination includes detailed verification of dental status and radiologic examination of the corresponding anatomical region. The third molars are the only teeth available for age estimation in target age spans around the age of 18 years for discriminating adults from minors [51, 66, 67]. Recent studies for discriminating adults and minors by using the third molar maturity index (I_{3M}) [68] showed 83 % correctly classified individuals with sensitivity and specificity of 70 and 98 %, respectively, when a specific cut-off value was used, which indicates better classification than the Demirjian staging system (DSS) [58]. Studies of I_{3M} on another Italian, Albanian, Croatian, and Brazilian sample confirmed the usefulness of I_{3M} and proposed a specific cut-off value for discriminating between being 18 years of age or not [64, 69–71]. However, the prevalence of missing, previously or intentionally extracted, or useless for assessment due to impaction with rotation or super positioning third molars, indicates the necessity for evaluating other possible anatomical structures for specific forensic or legal purposes [64, 72]. The other permanent teeth, the first seven in both jaws complete their development by 12 and 14 years of age and therefore are useless for discriminating between adults and minors [73–75]. Contrary to different dental methods, insights into x-ray of the knee joint and SKJ are available in each healthy individual in question.

Few other skeletal indicators have been tested for age estimation in adolescence. AGFAD recommended x-ray examination of the left hand in combination with a physical and dental examination [25]. According to some authors, bones of the hand and wrist are unusable for assessing adulthood, because their skeletal development may already be finished by the age of 16 in both sexes [3, 76, 77]. On the other hand, Garamendi et al. [24], on a sample of male Moroccan immigrants aged between 13 and 25 years, indicated usefulness of an atlas by Greulich and Pyle [78] on hand and wrist skeletal development and DSS on third molars in discriminating adults and minors. Garamendi et al. [24] also showed that combining results with both methods reduce the false positives or number of minors selected as adults, which is ethically unacceptable.

The lack of particular visible indicators in the cut-off age of majority excludes also cervical vertebrae, used for estimating of skeletal development for different orthodontic and other clinical dental purposes [79, 80]. Cameriere et al. [80] showed continuous development of the fourth cervical body only until 14 and 13 years in males and females, respectively, while Thevissen et al. [81] reported that the combination of the final third molar developmental stages and skeletal development of cervical vertebrae, which include the age span around 18 years of age, irrelevantly increases the accuracy of age prediction. Age prediction, based on a combination of third molars and cervical information of individuals older than 14 years, was even reduced [81]. However, any studies that would evaluate possible inclusions and combinations of all useful anatomical regions in determining the margin of error for age estimation,



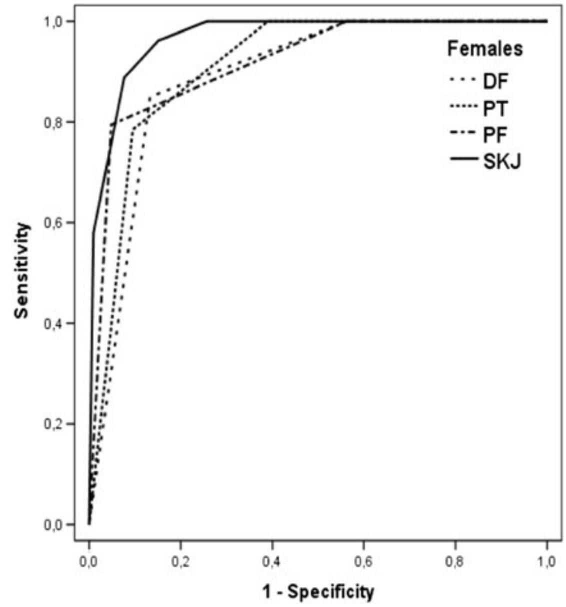


Fig. 5 Receiver operating characteristic curves for discriminating that males and females are 18 years of age and older or under 18 years for scoring maturation, from 0 to 2, of the distal femoral (*DF*), proximal tibial (*PT*), proximal fibular (*PF*) epiphyses and total score, from 0 to 6, of all three epiphyses at the knee joint (*SKJ*)

or answer the particular question, whether an individual is adult or minor, are welcome [24, 64]. Furthermore, if analysis of the hand found that development was completed, radiologic analysis of the medial clavicle was recommended [25]. The stages of ossification of cartilage of the sternal ends of the clavicle are difficult to identify indubitably on a conventional radiograph, with moderate examiner agreement [22]. Therefore, the radiographs of the clavicles are unsecure for reliable and updatable results that could be useful for age prediction within reliable confidence intervals [22, 82]. Computer tomography (CT) evidence of the medial clavicular epiphyseal cartilage is recommended for establishing whether an individual has attained 21 years of age [25]. Thin slice CTs established the possibility of substaging missing or partially epiphyseal cartilage ossification with a total of six substages, where the last substage 3c was only found at minimum age of 19 years, which may be suitable as a possible indicator of legal adult age [83–85].

Table 4 The area under the curve (AUC) of receiver operating characteristic curve that the participant is ≥18 years or <18 years of the distal femoral (DF), proximal tibial (PT), proximal fibular (PF) epiphyses and total score of maturation of the epiphyses at the knee joint (SKJ) in males (M) and females (F)

Score	Sex	AUC	95 %CI of AUC	SE
DF	M	0.944	0.914 to 0.974	0.016
	F	0.891	0.846 to 0.937	0.023
PT	M	0.962	0.941 to 0.983	0.011
	F	0.910	0.870 to 0.951	0.021
PF	M	0.974	0.959 to 0.990	0.008
	F	0.918	0.881 to 0.956	0.019
SKJ	M	0.991	0.984 to 0.998	0.004
	F	0.968	0.947 to 0.989	0.011

95CI of AUC 95 % confidence interval of AUC, *SE* standard error of AUC

Nowadays, age evaluation of young adults is of special interest in legal and forensic medicine and the accuracy of the methods has different success rates [2, 65]. In recent years, large illegal immigration, mainly from Northern, Sub-Sahara, and Eastern Africa as well as from Syria, Afghanistan, and Pakistan is occurring in Italy and other EU border countries [86–88]. Italy has a specific geographical location, located at the main point of entry for immigrants from these countries. According to the annual report by the Organization for Economic Cooperation and Development (OECD), which also includes detailed annual statistics on immigration, an increase in illegal immigrants between 2010 and August 2011 was almost 14-fold [87, 89, 90].

The migrants are fleeing from their country of origin for different reasons: war, famine, and economic and political motives [87]. A large number of illegal immigrants, asylum seekers, and unaccompanied minors are entering in target destinations without adequate identity documents due to many reasons and circumstances [89]. People without documents may become subjects to forced labor, recruited into the army, exploited, or forced into marriage in an attempt to obtain legal documents [64, 91]. It is particularly important that minors are ethically and properly treated and not wrongly classified as adults during administrative, investigative, or judicial proceedings [91]. A specific approach to assessing adulthood related to the level of probability of correct

Table 5 Values of 2-by-2 contingency tables describing discrimination performance of the different values of the total scores of the maturation of the epiphyses at the knee joint (SKJ) that the participant is ≥18 years or <18 years in males

Values	SKJ≥					
	1	2	3	4	5	6
TP	124	124	124	117	112	86
FP	61	45	22	5	1	0
FN	0	0	0	7	12	38
TN	49	65	88	105	109	110
Acc	0.74 (0.70 to 0.74)	0.81 (0.77 to 0.81)	0.91 (0.87 to 0.94)	0.95 (0.91 to 0.97)	0.94 (0.91 to 0.95)	0.84 (0.80 to 0.84)
p_1	1.00 (0.97 to 1.00)	1.00 (0.97 to 1.00)	1.00 (1.00 to 1.00)	0.94 (0.90 to 0.97)	0.90 (0.87 to 0.91)	0.69 (0.66 to 0.69)
p_2	0.45 (0.41 to 0.45)	0.59 (0.55 to 0.59)	0.80 (0.73 to 0.80)	0.96 (0.91 to 0.98)	0.99 (0.95 to 1.00)	1.00 (0.96 to 1.00)
J-index	0.45 (0.37 to 0.45)	0.59 (0.52 to 0.59)	0.80 (0.73 to 0.80)	0.90 (0.82 to 0.95)	0.89 (0.82 to 0.91)	0.69 (0.62 to 0.64)
PPV	0.67 (0.65 to 0.67)	0.73 (0.71 to 0.73)	0.85 (0.82 to 0.85)	0.96 (0.92 to 0.98)	0.99 (0.95 to 1.00)	1.00 (0.95 to 1.00)
NPV	1.00 (0.91 to 1.00)	1.00 (0.94 to 1.00)	1.00 (0.95 to 1.00)	0.94 (0.89 to 0.96)	0.90 (0.87 to 0.91)	0.73 (0.71 to 0.74)
LR+	1.80 (1.63 to 1.80)	2.44 (2.16 to 2.44)	5.00 (4.09 to 5.00)	20.76 (10.13 to 48.10)	99.35 (18.13 to 1910.68)	inf (17.41 to inf)
LR−	0.00 (0.00 to 0.08)	0.00 (0.00 to 0.06)	0.00 (0.00 to 0.04)	0.06 (0.03 to 0.10)	0.10 (0.09 to 0.14)	0.31 (0.31 to 0.35)
Bayes PTP	0.74 (0.69 to 0.79)	0.80 (0.74 to 0.86)	0.89 (0.83 to 0.95)	0.97 (0.91 to 1.00)	0.99 (0.93 to 1.00)	1.00 (0.94 to 1.00)

TP true positive values, *FP* false positive values, *FN* false negative values, *TN* true negative values, *Acc* accurate classification, p_1 sensitivity, p_2 specificity, *inf* infinity, *J-index* Youden index; *PPV* positive predictive value, *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR−* negative likelihood ratio, *Bayes PTP* Bayes posttest probability

classification of a subject also depends on the context, civil or criminal cases [92]. When the results obtained with a confidence interval that includes age and the age of consent, the ethical principle to be applied is the approach *in dubio pro reo* or benefit of the doubt [93]. Any method for age estimation that could reduce errors of misclassification is worthy of interest. When judging technically unacceptable errors (that subjects who are 18 years or older are classified as minors) against an ethically unacceptable error (that minor was classified as an adult), according to Garamendi et al. [24], the decision should be based on the benefit of the doubt [5, 64, 91, 94].

Table 6 Values of 2-by-2 contingency tables describing discrimination performance of the different values of the total scores of the maturation of the epiphyses at the knee joint (SKJ) that the participant is ≥18 years or <18 years in females

Values	SKJ≥					
	1	2	3	4	5	6
TP	107	107	107	103	95	62
FP	74	62	27	16	8	1
FN	0	0	0	4	12	45
TN	31	43	78	89	97	104
Acc	0.65 (0.61 to 0.65)	0.71 (0.67 to 0.71)	0.87 (0.83 to 0.87)	0.91 (0.86 to 0.93)	0.90 (0.85 to 0.94)	0.78 (0.74 to 0.79)
p_1	1.00 (0.96 to 1.00)	1.00 (0.96 to 1.00)	1.00 (0.96 to 1.00)	0.96 (0.91 to 0.99)	0.89 (0.84 to 0.92)	0.58 (0.54 to 0.59)
p_2	0.29 (0.26 to 0.30)	0.41 (0.37 to 0.41)	0.74 (0.70 to 0.74)	0.85 (0.80 to 0.87)	0.92 (0.87 to 0.96)	0.99 (0.95 to 1.00)
J-index	0.29 (0.22 to 0.30)	0.41 (0.33 to 0.41)	0.74 (0.67 to 0.74)	0.81 (0.71 to 0.86)	0.81 (0.71 to 0.88)	0.57 (0.48 to 0.59)
PPV	0.59 (0.57 to 0.59)	0.63 (0.61 to 0.63)	0.80 (0.77 to 0.80)	0.87 (0.82 to 0.89)	0.92 (0.87 to 0.96)	0.98 (0.91 to 1.00)
NPV	1.00 (0.87 to 1.00)	1.00 (0.90 to 1.00)	1.00 (0.95 to 1.00)	0.96 (0.90 to 0.99)	0.89 (0.84 to 0.92)	0.70 (0.67 to 0.70)
LR+	1.42 (1.29 to 1.42)	1.64 (1.53 to 1.69)	3.89 (3.25 to 3.89)	6.32 (4.54 to 7.76)	11.65 (6.50 to 22.56)	60.84 (10.00 to 1180.95)
LR−	0.00 (0.00 to 0.15)	0.00 (0.00 to 0.10)	0.00 (0.00 to 0.05)	0.04 (0.01 to 0.11)	0.12 (0.08 to 0.19)	0.42 (0.41 to 0.49)
Bayes PTP	0.70 (0.65 to 0.75)	0.73 (0.68 to 0.79)	0.86 (0.80 to 0.93)	0.91 (0.84 to 0.98)	0.95 (0.88 to 1.00)	0.99 (0.93 t0 1.00)

TP true positive values, *FP* false positive values, *FN* false negative values, *TN* true negative values, *Acc* accurate classification, p_1 sensitivity, p_2 specificity, *J-index* Youden index, *PPV* positive predictive value, *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR−* negative likelihood ratio, *Bayes PTP* Bayes posttest probability

Ethical aspects of age estimation also include reduction of unnecessary exposure or additional radiologic examination of a specific region. Effective doses, expressed in Sievert (Sv) units, indicate a possible damage from ionizing exposure. Absorbed doses are a physical quantity and represent the mean energy (Joule) imparted to matter per unit mass (kg) by ionizing radiation and expressed in Gray (Gy) units [41]. Absorbed doses can be estimated by anthropomorphic phantoms with dosimeters or by computer programs and are important for techniques that include high effective doses or expose sensitive tissues in the radiation beam [41]. Standard x-ray examinations have effective doses between 0.01 and 10 micro Sievert (μSv), while annual effective dose from background radiation is about 3 mSv [41]. Computer and direct digital radiography have the possibility of reducing both effective and absorbed doses. All personnel involved in diagnostics are obligated to balance risks and benefits of specific radiographic examination [5, 41]. Reported effective doses vary in literature. An average effective dose of 0.005 mSv for the x-ray of the knee is as low as 0.005 mSv for the intraoral x-ray, compared with 0.01 mSv for panoramic and 0.2 mSv for the dental CT [41]. Ethical issues related to age estimation procedures based on radiographic methods are especially relevant for increased migration, and further efforts are needed for higher homogenization and standardization [11, 27, 28, 91]. MRI of the knee is a non-invasive method for age estimation, without the disadvantage of radiologic examination. Jopp et al. [33] categorized the maturity of the right PT epiphysis by MRI into three stages, while Dedouit et al. [32] defined five MRI stages of maturation of the DF and PT epiphyses. Dedouit et al. [32] showed that by the presence of a continuous horizontal cartilage signal intensity between the metaphysis and the epiphysis at the knee, it is possible to distinguish growth plate patterns in an age range between 17 and 30 years for both genders. Krämer et al. [38] studied 290 MRI scans of males and females aged between 10 and 30 and showed that the final stage of ossification of PT epiphysis, or minimum limit of stage 4 (the epiphyseal cartilage is fully ossified) according to Schmeling et al. [95], does not occur before 18 years of age. Saint-Martin et al. [36] confirmed results of Krämer et al. [38] on 214 males aged between 14 and 20 years of age. Both Saint-Martin et al. [34–36] and Krämer et al. [38] have confirmed that MRI is a useful non-invasive technology for age estimation of adolescents and agreed that epiphyseal union at the knee occurs earlier in females than in males.

In conclusion, this is the first study that evaluated the efficacy and validity of different stages of ossification of epiphyses at the knee joint and SKJ scores in the different sample of anteroposterior x-rays with the modification of previously proposed SKJ scores to classify adults and minors. Principally, the evidence of an unfused epiphyses at the knee joint suggest being a minor while finished ossification of the knee suggests the attainment 18 years of age with high probability. The SKJ score can give additional information to dental status if radiographs of the knee exist, especially if the abovementioned was not possible to be obtained, i.e., missing or extracted tooth and if refusing the physical examination, when reporting specific legal or forensic questions including age classification [26, 64]. The authors recommend verification of the SKJ method on other reference samples in order to check its usefulness and possible ethnic, racial, and socioeconomic impacts on the recommended SKJ cut-off values.

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Compliance with ethical standards The protocol to collect X-rays and perform the study was conducted in accordance with the ethical standards laid down by the Declaration of Helsinki. The Ethics Committee for Research Involving Human Subjects of the Foligno Hospital approved the study [96].

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