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Age Estimation Based on Analyses of Sternal End of Clavicle and the First Costal Cartilage

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Procena godina starosti osobe analizom unutrašnjeg okrajka ključne kosti i hrskavice prvog rebra

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ABSTRACT

Age Estimation Based on Analyses of Sternal End of Clavicle and the First Costal Cartilage

In order to establish reliable age indicator in the period when all other epiphyseal age indicators have already been inactivated medial clavicle as the bone with the longest period of growth became the object of various investigations. However, the lack of population-specific method often made it unreliable in some population groups. The influence of socioeconomic conditions as well as interethnic and interracial variation on skeletal epiphyseal union phases has been acknowledged in scientific literature. Therefore, the importance of developing reliable population-specific age estimation methodology for the specific geographic region is outlined.

The ossification patterns of the first costal cartilage represent another interesting feature of the same anatomical region which according to its position in human body is accessible for examinations in living individuals who undergo conventional radiographic and computed tomography examinations in the same field of view as clavicle.

The broad range of macroscopic, histomorphometric and radiological analyses were conducted in order to establish the reliable age indicator. However, it remained unclear whether introduction of new more precise anatomical and other criteria in analyses of the medial clavicle and the first costal cartilage as well as their mutual analyses would be beneficial for age estimation. Therefore, this study encompassed our local population and was carried out with aim to examine whether morphological, radiological and histological analysis of medial clavicles as well as radiological examination of the first costal cartilage could be applied with success in age assessment of individuals.

The study was composed of two sections.

The first part of the study encompassed the collection of medial clavicles which was founded in cooperation of Laboratory for Anthropology, Institute of Anatomy with the Institute of Forensic Medicine, School of Medicine, University of Belgrade. The sample comprised 67 medial clavicles derived from individuals of different sex with age range

from 20 to 90 years. During the assessment of macroscopic morphological features the progress of epiphyseal union was recorded by applying a three-phase scoring system developed by Schaefer and Black. Further macroscopic analyses of the medial clavicles involved studies of the following morphological features: basic morphology, relief, porosity and shape of the articular surface, presence of ossific nodule, morphology of the articular surface margin, and morphology of the notch for the first costal cartilage. Subsequently, the conducted histological analyses of medial clavicles involved following bone histomorphometric parameters: cortical width, cancellous bone area/tissue area, and minimum trabecular width, in accordance with the standards of the American Society for Bone and Mineral Research.

The second part of the study data encompassed multislice computed tomography (CT) examinations of thoracic region of 154 patients in Institute for Oncology and Radiology of Serbia, aged between 15 and 35 years in the moment of performed diagnostic procedure. CT scan images allowed the assessment of the ossification status of medial clavicular epiphyseal cartilage following five-phase scoring system developed by Schmeling et al. and modified by Schulz et al., recording of thickness of the anterior and posterior cortex, enlargement of medullar canal, diameter of clavicular shaft and radiodensity of sternal epiphyseal-metaphyseal region. The assessment of the bony changes of anterior and posterior margin of costal face which extend through cartilage medially to sternum was carried out in concordance to published methodology of Moskovitch et al. The analyses of the first costal cartilage also made distinction in the sex, lateralization, number of present linear projections i.e. beaks, and radiodensity of costal cartilage.

According to the results, apart from the epiphyseal union timing, the macroscopic part of the current study managed to identify several other age-related features which could be applied as additional guidance for age estimation and that could be easily identified by naked eye. Among investigated morphological features, signs of lipping in the region of the notch for the first rib as well as exostoses and bone overgrowths of the articular surface margin should be considered as age-depended attributes of medial clavicles.

The results also suggested trabecular bone volume fraction and minimum trabecular width as age-distinctive microscopic features of medial clavicles in men.

The CT analyses of the sternal epiphyseal-metaphyseal region revealed significant correlation to age and led to the implementation of several age related equations. Beside radiodensity and stages of epiphyseal cartilage ossification of medial clavicles, the study also detected other age-related features among males, such as calculated anterior to posterior cortical thickness ratio or for instance, diameter of medullar canal and clavicular shaft.

Although the analyses of the first costal cartilage estimated statistically significant correlation between individual's age and calculated stages of the ossified and calcified linear projections (OCP), the distinction between stages was not satisfying.

The interaction between applied scoring systems for the ossification status of medial clavicular epiphyseal cartilage and OCP's stages were not statistically significantly influenced by age, and their mutual enrollment might not be advisable. However, the results of the current study suggested that acquired radiodensity of sternal epiphyseal-metaphyseal region and radiodensity of the first costal cartilage stand out as an interesting new age predictors with mutual relationship which could represent a usuful additional tool in future analyses.

Evaluation of sex differences in the observed macroscopic features revealed that epiphyseal union timing, morphology of the notch for the first rib, margin of the articular surface, and basic morphology of articular surface were sex-depended attributes. Moreover, sex difference was ascertainable in two microscopic characteristics: trabecular bone volume fraction and minimum trabecular width. Intersex variability was also observed by CT in several age-related features: calculated anterior to posterior cortical thickness ratio, diameter of medullar canal, and diameter of clavicular shaft. Finally, the current study identified females' tendency towards the higher epiphyseal union stages.

Age estimation applied in post-mortem as well as in living is essential in identification process and in cases of misidentification. Therefore, the importance of developing reliable population-specific age estimation methodology is emphasised. Unfortunately, a few methods are tested and applied in our region. In fact, this is the first study that has been carried out in a Balkan population with aim to examine whether morphological, radiological and histological analysis of medial clavicles as well as radiological

examination of the first costal cartilage could be applied with success in age assessment of individuals in anthropological and forensic practice. The study also identified other discerning morphological macroscopic and microscopic as well as radiological features in the region of the first costal cartilage and medial clavicle that correlated with age, and could be applied as additional guidance for age estimation in each specific case.

Keywords: clavicle, the first rib, costal cartilage, macroscopic, histomorphometric, radiological, CT, sex, age assessment.

RESEARCH FIELD: MEDICINE – Skeletal Biology.

REZIME

Procena godina starosti osobe analizom unutrašnjeg okrajka ključne kosti i hrskavice prvog rebra

U odnosu na druge kosti ljudskog skeleta, epifiza unutrašnjeg okrajka ključne kosti poslednja je koja vremenom srasta sa dijafizom. Zato unutrašnji okrajak ključne kosti jeste jedinstvena struktura koja može da predstavlja pouzdan pokazatelj starosti osobe u trenutku kada su svi ostali epifizni nastavci srasli sa dijafizama. Smatra se da na proces epifizno-dijafiznog spajanja utiču kako socioekonomski uslovi života, tako i rasna i etnička pripadnost. Zbog toga je značajno razvijanje pouzdanog populaciono-specifičnog metoda za procenu godina starosti osoba u specifičnom geografskom regionu.

Proces osifikacije rebarne hrskavice prvog rebra predstavlja još jednu karakteristiku istog regiona tela, koji se zahvaljujući svojoj specifičnoj anatomskoj lokalizaciji može prikazati i analizirati tokom radiografisanja i pregleda metodom kompjuterizovane tomografije (CT), i to u istom polju pregleda gde i ključna kost.

Širok spektar makroskopskih, histomorfometrijskih i radioloških ispitivanja sproveden je da bi se utvrdili pouzdani pokazatelji godina starosti osobe, ali još uvek nisu formirani novi i dovoljno precizni anatomski i drugi kriterijumi koji bi obuhvatili kako unutrašnji okrajak ključne kosti, tako i prvu rebarnu hrskavicu, kako svaku strukturu samu za sebe, tako i u kombinaciji, a koji bi omogućili bolje određivanje starosti osoba. Stoga je ova studija obuhvatila našu populaciju i ispitala da li morfološke, radiološke i histološke karakteristike unutrašnjeg okrajka ključne kosti, kao i radiološke karakterisike prve rebarne hrskavice, mogu pouzdano da posluže pri proceni godina starosti neke osobe.

Studija se sastoji iz dva dela.

Prvi deo studije obuhvata analizu kolekcije unutrašnjih okrajaka ključnih kostiju koja je nastala u saradnji Laboratorije za antropologiju Instituta za anatomiju sa Institutom za sudsku medicinu, Medicinskog fakulteta Univerziteta u Beogradu. Uzorak je bio sačinjen od 67 ključnih kostiju osoba oba pola i starosti od 20 do 90 godina. Za utvrđivanje makroskopskih morfoloških karakteristika razvoja epifizno-dijafiznog spoja

primenjen je trostepeni skor-sistem po Schaefer-Black-u. Makroskopske analize uzorka obuhvatile su i proučavanje sledećih morfoloških odlika medijalnog okrajka ključne kosti: bazična morfologija, reljef, poroznost i oblik zglobne površine, prisustvo koštane kvržice, morfologija ivice zglobne površine i morfologija useka za hrskavicu prvog rebra. Histološke analize unutrašnjih okrajaka ključnih kostiju obuhvatile su merenje: debljine korteksa, odnosa između površine spongiozne kosti i površine tkiva na preparatu, kao i minimalne širine trabekula. Ove histomorfometrijske karakteristike standardizovao je American Society for Bone and Mineral Research.

Drugi, klinički deo studije činili su MSCT ("*multislice computed tomography*") pregledi grudnog koša 154 pacijenta koji su pregledani na Institutu za onkologiju i radiologiju Srbije, starosti između 15 i 35 godina života. Na osnovu CT snimaka izvršena je procena stepena osifikacije epifizne hrskavice unutrašnjeg okrajka ključne kosti koristeći petostepeni skor-sistem po Schmeling-u koji je modifikovao Schulz, merenje debljine prednjeg i zadnjeg korteksa kosti, širine medularnog kanala, dijametra tela ključne kosti i stepena apsorpcije rendgenskih zraka u predelu sternalnog epifizno-metafiznog regiona. Na CT snimcima, analizirane su i koštane promene u predelu prednje i zadnje ivice prednjeg okrajka rebra koje se pružaju kroz rebarnu hrskavicu ka grudnoj kosti. Gradacija ovih promena izvršena je po Moskovitch-u. Analize prve rebarne hrskavice obuhvatile su i: pol i godine starosti ispitivane osobe, broj izraštaja koji se pružaju od prednje i zadnje ivice prednjeg okrajka prvog rebra kroz hrskavicu ka grudnoj kosti i stepen njihove izraženosti sa leve i desne strane tela, kao i stepen apsorpcije rendgenskih zraka u predelu rebarne hrskavice.

Na osnovu sprovedenih analiza, makroskopski deo studije je osim razvoja epifizno-dijafiznog spoja otkrio još nekoliko karakteristika koje zavise od godina starosti, a koje se mogu upotrebiti kao dopuna pregledu obzirom da su lako uočljive. U okviru analiziranih morfoloških odlika, dobnu zavisnost je pokazala pojava lipinga u predelu useka za hrskavicu prvog rebra, kao i egzostoze i koštani izraštaji ivice zglobne površine ključne kosti.

Rezultati su, takođe, ukazali da površina koštane frakcije spongiozne kosti, kao i minimalna širina trabekula, predstavljaju histomorfometrijske parametre koji se menjaju sa godinama kod muškaraca.

Analiza CT snimaka unutrašnjeg epifizno-metafiznog regiona ključnih kostiju ustanovila je više dobno zavisnih parametara, čiji je odnos prikazan u izvedenim jednačinama. Osim stepena apsorpcije rendgenskih zraka i stepena osifikacije epifizne hrskavice unutrašnjeg okrajka ključne kosti, značajnu korelaciju sa godinama života kod muškaraca pokazao je i indeks debljine prednjeg i zadnjeg korteksa kosti, širina medularnog kanala, kao i dijametar tela ključne kosti.

Iako su analize hrskavice prvog rebra dokazale značajnu statističku korelaciju između godina starosti i procenjenog stepena koštanih promena hrskavice, mogućnost međusobnog diferenciranja različitih faza starosti bila je nezadovoljavajuća.

U slučaju zajedničke primene sistema skorovanja stepena osifikacije epifizne hrskavice unutrašnjeg okrajka ključne kosti i stepena koštanih promena prve rebarne hrskavice, procenjena interakcija nije pokazala značajnu statističku korelaciju sa godinama starosti, te ovakav način pregleda nije zadovoljavajući. Međutim, zajednička primena stepena apsorpcije rendgenskih zraka unutrašnjeg okrajka ključne kosti i prve rebarne hrskavice, istakla se kao značajan novo-otkriveni pokazatelj godina starosti koji može predstavljati koristan dopunski pregled u budućnosti.

Analiza pregledanih makroskopskih morfoloških odlika otkrila je polne razlike u vremenskom razvoju epifizno-dijafiznog spoja, morfologiji useka za hrskavicu prvog rebra, kao i morfologiji ivice zglobne površine i bazičnoj morfologiji zglobne površine. Razlike u polu su bile uočene i u histološki analiziranoj površini koštane frakcije spongiozne kosti, kao i kod minimalne širine trabekula. Rezultati kliničkog dela studije su na osnovu CT snimaka ukazali na polne razlike pojedinih dobno zavisnih karakteristika, kao što su: indeks debljine prednjeg i zadnjeg korteksa kosti, širina medularnog kanala, kao i dijametar tela ključne kosti. Sprovedeno istraživanje je utvrdilo tendeciju ženskih osoba ka višim stadijumima osifikacije epifizne hrskavice unutrašnjeg okrajka ključne kosti.

Utvrđivanje godina starosti žive ili mrtve osobe jeste bitno kod osoba koje nisu identifikovane ili u čiji se identitet sumnja, kako s medicinsko-forenzičkog, tako i jurističkog aspekta. Preciznost i pouzdanost svake stare ili novouvedene metode za procenu godina starosti neke osobe, u vezi je kako sa geografskim poreklom osoba koje su činile uzorak na kojem je metoda razrađena, tako i sa njihovim socio-ekonomskim

stanjem i položajem. Kod nas, nažalost, postoji malo ovih metoda i slabo su razrađene, pa se i ne primenjuju. Zapravo, ova studija je prva koja je u populaciji balkanskog regiona testirala primenljivost i ponovljivost postojećih razrađenih metoda. Takođe, ovom studijom ustanovljene su i druge morfološke – kako makroskopske, tako i mikroskopske – karakteristike, ali i rendgenološke odlike unutrašnjeg okrajka ključne kosti i hrskavičavog dela prvog rebra, koje kako same za sebe, tako i u kombinaciji, mogu biti od koristi za određivanje životne starosti svake konkretno ispitivane osobe.

Ključne reči: ključna kost, prvo rebro, rebarna hrskavica, makroskopski, histomorfometrijski, radiološki, CT, pol, utvrđivanje starosti.

NAUČNA OBLAST: MEDICINA – Biologija skeleta.

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Introduction

Age estimation represents one of the main objects in the field of forensic practice and physical anthropology. Throughout the literature scholars outlined different skeletal markers that could be considered as age-specific. Therefore, the thoroughly conducted macroscopic analyses of skeletons led to development of several discerning age-related patterns mostly emphasising morphological changes observed in cartilaginous joints in the body mid-line such as pubic symphyses and sternal end of the ribs (1, 2). Nevertheless, the existing distinction between the first and lower ribs was acknowledged, as well (3, 4). Certainly, the sternal clavicular end also represented an interesting object of numerous investigations.

Contrary to pubic symphysis, clavicles could not be associated with physical stress. Namely, clavicles are engaged in locomotion just occasionally; they could not be put in a correlation with pregnancy neither associated with weight bearing. However, in the lower costal cartilages, the influence of respiratory movements was acknowledged (3, 4). Thus, it is considered that the first cartilage is only affected by extreme physical stress on the upper chest (3).

After excluding involvement of physical stress, the anatomical features of clavicles could embrace full scientific focus. The medial clavicular epiphysis is the last to fuse among all long bones (5, 6). Thus, with the longest period of growth the medial clavicle retains particular opportunity to become reliable age indicator in the period when all other epiphyseal age indicators have already been inactivated.

Therefore, since the nineteenth century clavicles seized interest of scholars. According to the available literature (7), medial clavicles were introduced in the field of age analyses in the year of 1871 by Henle. Furthermore, the activities of various authors are reviled at the early years of the twentieth century (7). The literature (7-9) especially outlined the Stevenson's publication in the year of 1924 (6) as well as Todd and D'Errico study (1928) which were both conducted on samples derived from American population i.e. Western Reserve collection. Afterwards, the complete collection became worldwide known as Hamann-Todd collection. The authors of both studies developed their own four-phase scoring methodologies which mutually slightly differed.

In the following years, the Todd and D'Errico's scoring system sustained the first modification in the study of McKern and Stewart (1957) who extended it to five-phase (7). Furthermore, the same survey for the first time proposed the mechanism of epiphyseal fusion with provided description of the beginning in a very centre of the epiphyseal face progressing to the superior margin and spreading in such manner to leave the inferior margin as a finale site of fusion (7). Finally in 1985, contemporary American population of both sexes were analysed by Webb and Suchey (9). Overall, the last modification of scoring system was conducted by Schaefer and Black in 2007 (10). It was believed that the reduction of McKern and Stewart's five-phase to a three-phase scoring system will reduce subjective error which was brought up by multiphased methodology (10).

At the turn of the twentieth and the beginning of the twenty first century several comprehensive macroscopic studies were published encompassing different populations (5, 10-12). In fact, the influence of socioeconomic conditions on skeletal epiphyseal union phases was recently emphasised as more important than interethnic variation (8, 13). The recent require of post-mortem age determination was accompanied by a growing demand for age estimation of living individuals in regard to legal relevance of genuine age in criminal, civil and asylum proceedings as well as in children from war-affected regions. Therefore, the importance of developing reliable population-specific age estimation methodology for the Balkan region was outlined with processes of forensic identifications which followed the years of war during the last decade of past century encompassing the region of former Socialist Federal Republic of Yugoslavia (12, 14). Moreover, criminal and asylum issues in the Europe Union (EU) not so rarely enroll individuals from Balkan populations (15).

Therefore, the recommended process of age assessment of young individuals involved physical and dental examinations with radiographic analyses of dentition and left hand (16, 17). The same authors considered additional examination of the medial clavicle as the most appropriate since independently from the anatomical approach to age estimation, radiological methodology was developed. The criteria were based on an official publication adopted in 2000 by the members of the *Study Group on Forensic Age Diagnostics of the German Association of Forensic Medicine – Arbeitsgemeinschaft für Forensische Altersdiagnostik, AGFAD* (17). However, the initially established conventional radiology methods and their impediments were gradually replaced by Computed Tomography (CT) analyses. The implementation of new methodology was initiated in 1998 by Kreitner et al. (18).

Heretofore, process of age estimation apart from macroscopic and radiological based studies involved also histological methods which were predominantly based on the life cycle of cortical bone (19, 20). Although previously applied in various skeletal sites, the assessment of the age-related deterioration of bone micro-architecture particularly encompassed femur and vertebrae (21, 22). Stout and Pane (19, 23) for the first time used analyses of osteon population densities in the clavicular mid-shaft in the process of age estimation.

The ossification patterns of the first costal cartilage represent another interesting feature of the same region that arises around puberty and develops mostly through the third and fourth decade of life (3, 16). In addition, its position in human body makes it accessible for examinations in living individuals who undergo conventional radiographic and CT examinations in the same field of view as clavicle (16, 24). However, while clavicles remain accessible in cadaveric material, the subtle changes of the first cartilage could be altered during preparation and would be certainly lost during deterioration process. According to the literature, the analyses of the ribs for the first time involved modern radiological techniques in 2010 when Moskovitch et al. (24) studied the age related changes of the first rib and its cartilage using multislice computed tomography (CT).

Finally, the broad range of macroscopic, histomorphometric and radiological analyses were conducted in order to establish the reliable age indicator mainly in individuals under 35 years of age (15, 25). Thus, the most of this methodology was based on determination of defining features for age legality boundary (25). However, it remained unclear whether introduction of new criteria and anatomical features in analyses of the medial clavicle and the first costal cartilage as well as their mutual analyses can be beneficial for discriminating between different age categories.

Research Goals

In order to investigate the relationship between analysed features and age, the specific aims of the current research were:

1) To observe macroscopic morphological features and histomorphometric parameters of medial clavicles and analyse their relation to age.
2) To assess the relation of multislice computed tomography appearances of medial clavicles and age.
3) To examine whether quantity and morphological appearances on multislice computed tomography images of the calcifications in the first costal cartilage correlate to age.
4) To examine mutual relation between the progress of epiphyseal union of medial clavicle and ossification pattern observed in the first costal cartilage.

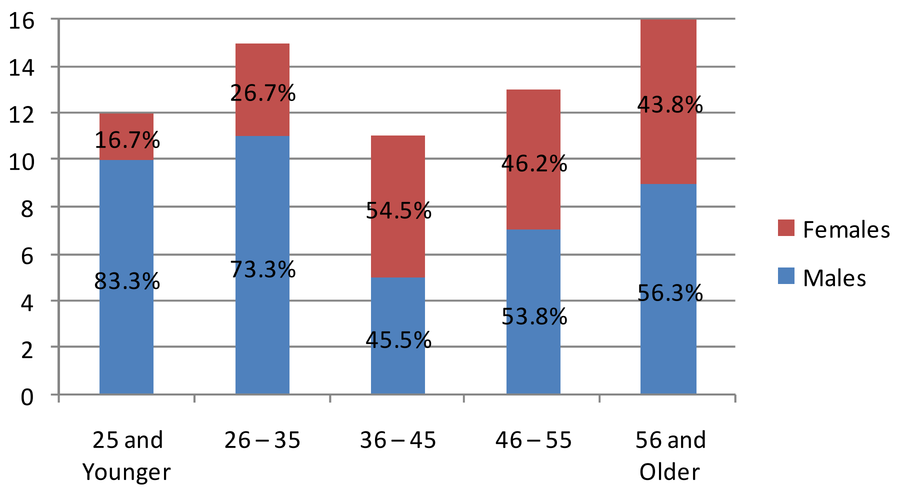
Material and Methods

The age estimation analyses involved two sample collections. Therefore, the study was composed in two sections. The first, Laboratory study, was conducted in Laboratory for Anthropology, Institute of Anatomy, School of Medicine, University of Belgrade, while the second, Clinical part was carried out in Institute for Oncology and Radiology of Serbia. All encompassed individuals belonged to contemporary Serbian population and their socioeconomic status was considered in boundaries of upper-middle-income country (26) with high human development index (27).

Laboratory Study

The first part of the study encompassed the collection of medial clavicles which was founded in cooperation of Laboratory for Anthropology, Institute of Anatomy with the Institute of Forensic Medicine, School of Medicine, University of Belgrade. The medial clavicular specimens were collected from individuals autopsied in the period from 1998 to 2001 at the Institute of Forensic Medicine. The exclusion criteria involved the presence of any visible deformity, signs of pathological conditions or fracture of the clavicle. The encompassed collection was comprised of 67 medial clavicles. Thus, the study sample was constituted of 42 (62.7%) males and 25 (37.3%) females (Chart 1). The data about sex and age of the individuals were derived from the death certificates. The age at death ranged from 20 to 90 years.

Chart 1. Age and sex distribution of individuals within the sample encompassed with the Laboratory study.



The results were presented and analysed in accordance to the five age groups. Therefore, the individuals aged 25 years and younger as well as those who were 56 and older were classified in two separate terminal groups, while the rest of the sample was sorted into three age groups with ten-year interval.

Each specimen was subjected to analyses of macroscopic morphological features of the articular surface.

During the assessment of macroscopic morphological features the progress of epiphyseal union was recorded by applying a three-phase scoring system which was developed by Schaefer and Black (10). A “non-union” phase was assigned in cases without traces of fusion (Fig. 1a). The second one, marked as “partial union” phase, was recorded if the signs of active union were observed followed by an epiphyseal line in the process of obliteration (Fig. 1b), often creating characteristic picture of “epiphyseal flake” (Fig. 1c). The final phase (“complete union”) was recognized by obliterated line of fusion (Fig. 1d), although signs of an epiphyseal scar might still be visible.

Further macroscopic analyses of the specimens involved observation of the following morphological features:

1. Basic morphology of the articular surface (convex, plane, concave) (Fig. 1d, 1i and 1j)
2. Relief of the articular surface (smooth, rugged) (Fig. 1e and 1f)
3. Porosity of the articular surface (not present, present) (Fig. 1e and 1g)
4. Shape of the articular surface (triangular, round, oval) (Fig. 1e, 1f and 1g)
5. Presence of ossific nodule (not present, present) (Fig. 1g)
6. Morphology of the margin (obtuse, sharp, with lipping, with exostoses and bone overgrowths) (Fig. 1d, 1j and 1l)
7. Morphology of the notch for the first costal cartilage (without outstanding margins, presence of outstanding margins, with lipping) (Fig. 1i, 1j and 1k).

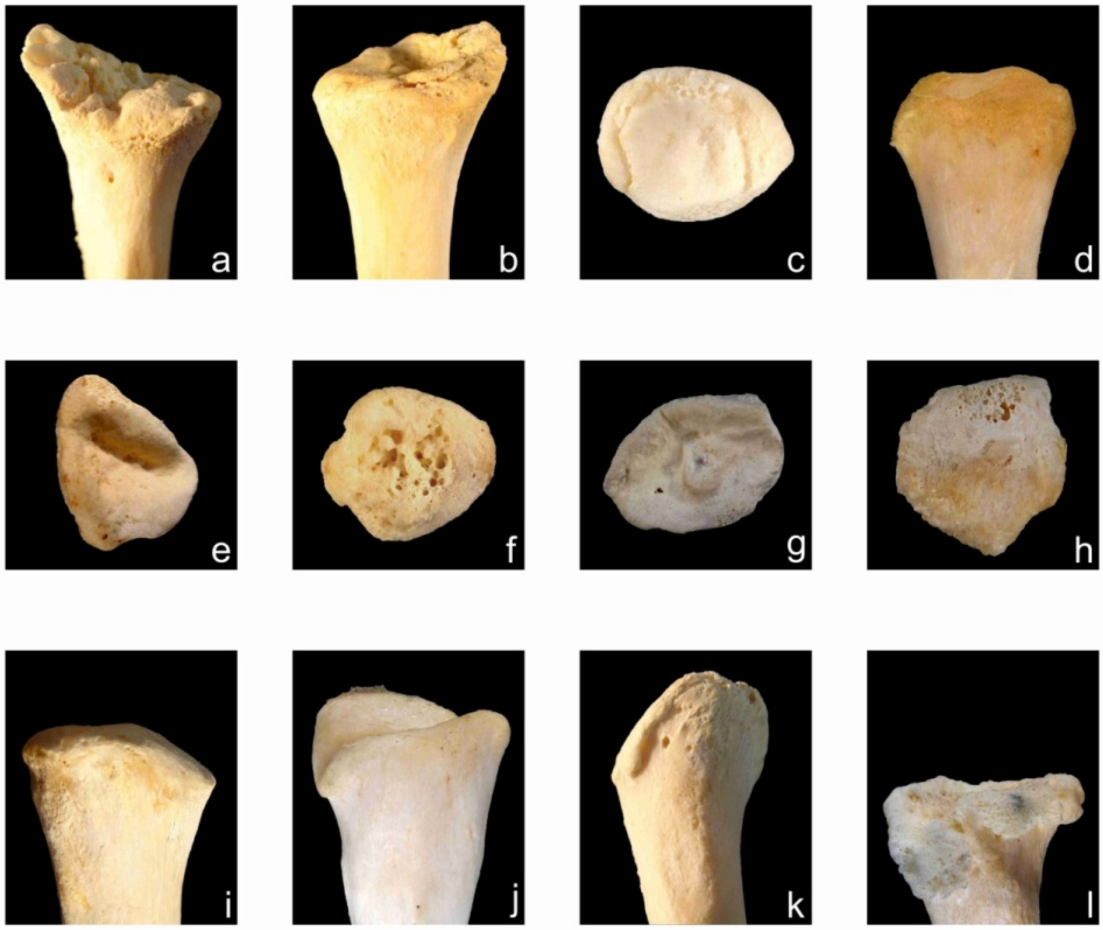


Fig. 1 Analised macroscopic morphological features of medial clavicle:

a) "Non-union" phase.
b) "Partial union" phase.
c) "Partial union" phase with characteristic picture of "epiphyseal flake".
d) "Complete union" phase with visible convex articular surface and obtuse margins.
e) Smooth relief, triangular shape with no signs of porosity of the articular surface.
f) Rugged relief and round shape of the articular surface.
g) Oval shape of the articular surface with present ossific nodule.
h) Round shape with present porosity of the articular surface.
i) Plane articular surface without outstanding margins in the region of the notch for the first rib.
j) Concave articular surface with outstanding margins in the region of the notch for the first rib and sharp margins of the articular surface.
k) Lipping in the region of the notch for the first rib.
l) Margins of the articular surface with exostoses and bone overgrowths.

After completing macroscopic morphological observations, histological analyses were performed on 11 male and 11 age-matched female specimens in Laboratory for Anthropology, Institute of Anatomy. The sample size was estimated according to the calculations performed in MedCalc software for statistical power of almost 80% and probability of Type I error of 0.05 and for expected correlation coefficient (23, 28). The specimens were embedded in epoxide resins (Mecaprex KM-U) and cut using water-cooled diamond-saw microtome (Leica SP1600) to 70μm cross-sections at the level positioned 5mm from the lower edge of the articular surface. Subsequently, the images of three low-power fields of each prepared specimen were acquired by Leica camera using Leica polarizing microscope. The fields were chosen randomly since field to field individual histological variation in the clavicle's sections was less expressed than in other long bones, as reported previously (23, 28). All of the obtained images (Fig. 2a and 2b) were analysed using KVI-POPOVAC V 2.2 software. The imaging system was calibrated by measuring a 1mm division scale.

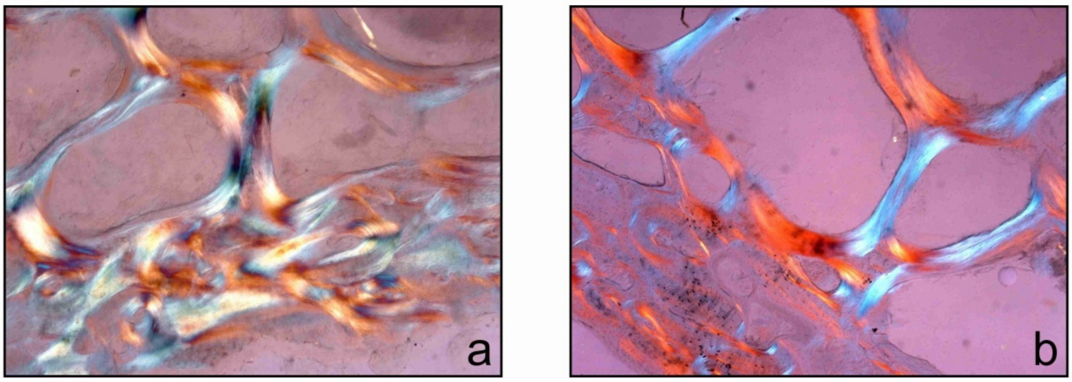


Fig. 2 Images of low-power fields of medial clavicles' histological cross-sections in young (a) and old (b) individuals.

The following bone histomorphometric parameters were calculated: cortical width (Ct.Wi), cancellous bone area/tissue area (Cn.B.Ar/T.Ar), and minimum trabecular width (Tb.Wi), in accordance with the standards of the American Society for Bone and Mineral Research (29, 30). The bone area and the tissue area were detected in the image processing software. The cortical width and minimum trabecular width were measured at ten randomly determined sites and average values for all acquired measurements were calculated for each individual.

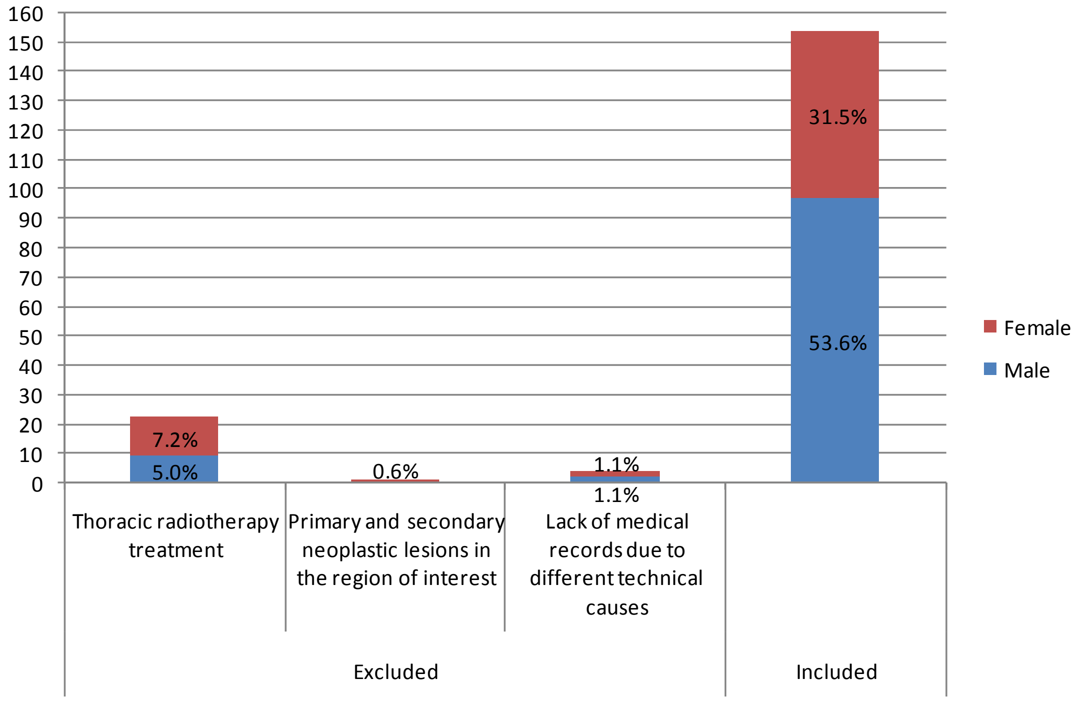
Clinical Study

The second part of the study data was derived from picture archiving and communication system (Carestream Health PACS) and medical archives of Institute for Oncology and Radiology of Serbia. The process of patient selection upon defined inclusion and exclusion criteria was based on data provided in medical records of each patient. The inclusion criteria for this study represented CT examination of thoracic region, and age of the patient in the range between 15 and 35 years in the moment of performed diagnostic procedure. The exclusion criteria encompassed involvement of hormone therapy, thoracic radiotherapy treatment, primary and secondary neoplastic lesions in the region of interest, presence of any visible deformity or signs of trauma of the clavicle, first rib or manubrium sterni.

The sample was selected from the group of patients who have undergone diagnostic CT examination in the period between 1st January 2011 and 31st December 2012; and therefore encompassed 181 patients.

The exclusion criteria disqualified from further analyses 27 patients (15%) in regard to thoracic radiotherapy treatment (12.2%), primary and secondary neoplastic lesions in the region of interest (0.6%), and lack of medical records due to different technical causes (2.2%). Therefore, the functional sample size for clinical study analyses comprised clavicles of both sides derived from CT examinations of 154 patients, whereof 97 (53.6%) were males and 57 (31.5%) females (Chart 2).

Chart 2. Age and sex distribution of individuals encompassed with the Clinical study.



The study data were comprised of CT examinations performed by Siemens SOMATOM Sensation Open. The included CT scans involved contrast-enhanced 3mm-reconstructed images from 5mm thickness. Each clavicle and first costal cartilage was subjected to analyses of discerning radiographic appearances. The analyses process was conducted on SIEMENS Syngo MultiModality Leonardo Workplace.

CT scan images allowed the assessment of the ossification status of medial clavicular epiphyseal cartilage following five-phase scoring system developed by Schmeling et al. (25) and modified by Schulz et al. (31). According to the provided instructions if ossification centre was not ossified, the first stage was assigned (Fig. 3a). Presence of ossification centre with unossified epiphyseal cartilage was considered as the second stage (Fig. 3b). The third stage stood for partly ossified epiphyseal cartilage (Fig. 3c). If epiphyseal cartilage was fully ossified and epiphyseal scar still visible, the fourth stage was assigned (Fig. 3d). However, fully ossified epiphyseal cartilage with no longer visible epiphyseal scar represented the fifth stage (Fig. 3e).

The clavicular analyses also involved direct recording of the following measurements: radiodensity of the sternal epiphyseal-metaphyseal region (Ep.Dn), thickness of the anterior ($Ct.Th_{ant}$) and posterior cortex ($Ct.Th_{post}$), diameter of the medullar canal (Ca.Wi), and clavicular shaft diameter (Dp.Wi) (30, 32, 33). Subsequently, acquired data permitted calculation of the following derived indices: anterior to posterior cortical thickness ratio ($Ct.Th_{ant}/Ct.Th_{post}$), as well as medullar to shaft diameter ratio (Ca.Wi/Dp.Wi). The measurements of clavicular diaphysis were obtained in the middle third of the shaft, always recording of all data at the same site of one clavicle. The radiodensity of the sternal epiphyseal-metaphyseal region was taken by manually shape-adjusted freehand ROI tool. For all recorded features the expression of sexual and side dimorphism was analysed as well.

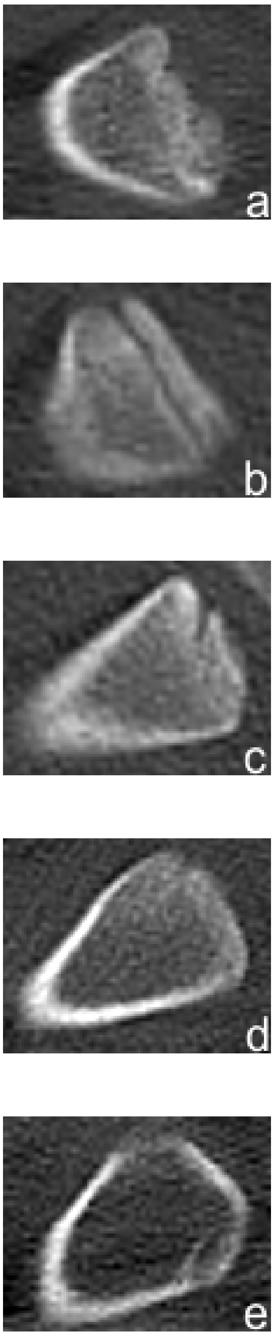


Fig. 3 CT scan images of the ossification status of medial clavicular epiphyseal cartilage:

a) the first stage,

b) the second stage,

c) the third stage,

d) the fourth stage,

e) the fifth stage.

Fig. 4 The CT image of the first cartilage stage.

This study also examined whether morphological appearances of the calcifications in the first costal cartilage correlate to age. The assessment of the bony changes of anterior and posterior margin of costal face which extend through cartilage medially to sternum was carried out in concordance with published methodology of Moskovitch et al. (24). Hence, the first stage was obtained in cases with no signs of the beaks or calcifications projecting medially in the costal cartilage (Fig. 4).

The second and third stage was assigned in the cases of ossified and calcified linear projections (OCP). Thus, the second stage was recorded if projection's length (OCP.Le) did not exceed 50% of distance between costal face and sternum (CS.Le) (Fig. 5), otherwise it was marked as the third stage (Fig. 6).

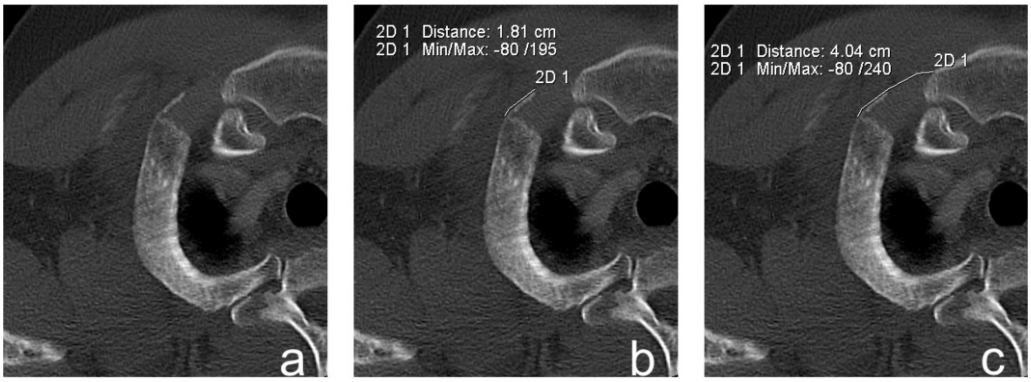


Fig. 5 The CT images of the second cartilage stage: a) image without measures, b) OCP.Le, c) CS.Le.

Fig. 6 The CT images of the third cartilage stage: a) image without measures, b) OCP.Le, c) CS.Le.

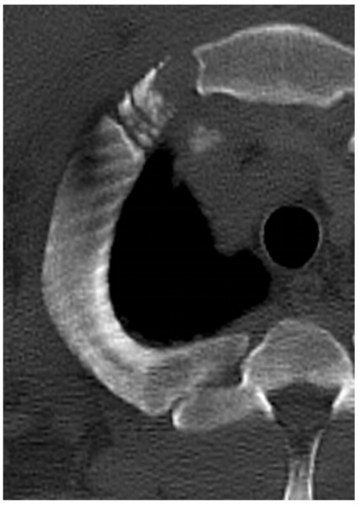


Fig. 7 The CT image of the fourth cartilage stage.

However, all non-linear appearances of calcifications projecting medially toward sternum were ascribed to the fourth stage (Fig. 7).

In addition, all data were recorded in adjusted axial plane of multi planar reconstruction viewer window in order to perform the most objective measuring in the plane of the first cartilage. Also, the projection's length was measured by manually shape-adjusted 2D freehand distance tool.

The analyses also made distinction in the sex, lateralization, number of present linear projections i.e. beaks, and radiodensity of costal cartilage (FC.Dn) which was recorded by manually shape-adjusted freehand ROI tool.

Statistical Analyses

The results were presented in the form of absolute and relative numbers distributed in the tables and charts, and followed with appropriate discussion. The conducted descriptive statistics measured central tendency of obtained data by mean values, while dispersion was quantified by standard deviation, standard error, minimum and maximum data values.

During the analyses, the normality of data distribution was assessed by Kolmogorov-Smirnov test. Subsequently, the parametric or non-parametric tests were applied in order to achieve research goals.

Thus, the Pearson's Chi-Square test was used for comparing non-parametric features between different age groups. The relation between obtained parametric data and individual's age was tested by Linear regression analysis while Spearman correlation was applied in the case of non-Gaussian distribution. The assessment of relation between obtained ordinal data and age involved Analysis of Variance (ANOVA).

The discrepancy between body sides among recorded parametric data was identified by Paired Student's t-test, while the discordance between non-parametric data was examined by Wilcoxon Test.

The estimation of sexual dimorphism involved Unpaired Student's t-test in cases of parametric data, while concerning non-Gaussian distribution Mann-Whitney U test was applied. The evaluation of intersex relations among ordinal data was conducted by Ordinal Regression analyses.

Overall, if the data expressed inter-sex variation, the results were presented independently for male- and female-sample part. If not, the sex differentiation was disregarded. Similarly, if the conducted analyses determined bilateralism, the results for both body sides were given. Otherwise, the presented results were without side differentiation.

The recordings were performed by two independent observers. The first observer was the author, while the second observation was conducted by the radiology specialist with previous experience in the field of forensic anthropology. The both observers were ignorant of individual's age and sex. The Cohen's Kappa test was used for assessment of interobserver reliability of observed macroscopic features of medial clavicles and macroscopically estimated epiphyseal union stages as well as radiologically analysed stages of the medial clavicle and the first costal cartilage. The instructions were provided by Landis and Koch (34). The Interclass correlation coefficient was applied in order to evaluate reproducibility of quantitative measurements made by different observers.

Statistical analyses were performed using SPSS for Windows, version 15. In all analyses, the significance level was set at 0.05.

Results

Laboratory Study – Macroscopic Analyses

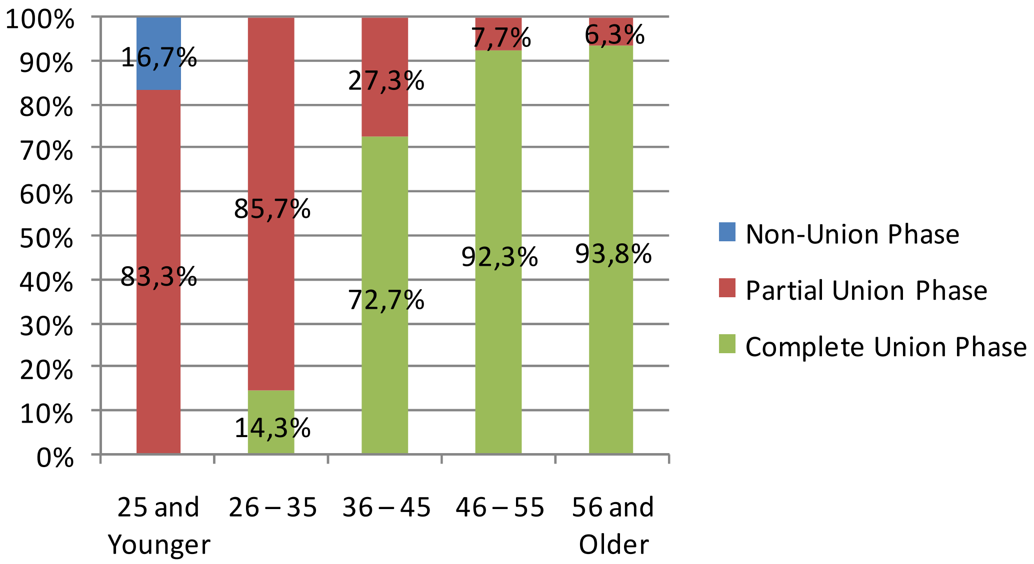
The macroscopic morphological analysis of epiphyseal union (Table 1 and Chart 3) determined “non-union” phase in only two subjects aged 20 and 22 years. Conversely, the features of “partial union” were recorded in all age groups, but predominantly in individuals of first two categories (81.5%). The youngest individual with this phase was 21 years old, while it had also been detected in a 65-year-old individual. In addition, only two recordings were made after 46 years of age (7.4%). Morphological characteristics of “complete union” were noticed in majority of individuals older than 36 years (94.6%). The earliest detection was at the age of 31. Statistical analysis confirmed significant difference in stage of epiphyseal union between different age groups, either in the whole sample ($p<0.001$) or separately the males ($p<0.001$) and females ($p=0.001$). Furthermore, females showed noticeable tendency towards higher epiphyseal union stages than the males of the same age ($OR=0.43$, $p=0.023$).

Table 1. Distribution of individuals by the age category and the progress of epiphyseal union.

AGE CATEGORIES	NON-UNION PHASE		PARTIAL UNION PHASE		COMPLETE UNION PHASE	
	n	%	n	%	n	%
25 AND YOUNGER	2	100.0	10	37.0	0	0
26 – 35*	0	0	12	44.4	2	5.4
36 – 45	0	0	3	11.1	8	21.6
46 – 55	0	0	1	3.7	12	32.4
56 AND OLDER	0	0	1	3.7	15	40.5
TOTAL	2	100.0	27	100.0	37	100.0

* The damage of the medial clavicular end excluded one individual from this analysis.

Chart 3. Distribution of epiphyseal union progress by the age category.



The significant difference in morphology of the notch for the first rib (p=0.002), and the margin of the articular surface (p=0.001), were verified between age groups within the whole sample. Namely, morphological analyses allowed detection of lipping in the region of the notch for the first rib (Table 2a) in individuals above 44 years of age, while the absence and presence of the signs of outstanding margins in the same region were recorded at much younger age (21 and 22 years, respectively). The youngest individual with observed exostoses and bone overgrowths of the articular surface margin (Table 2b) was 53 years old. On the contrary, the earliest signs of obtuse and sharp clavicular margins were recorded at the age of 21. However, these age-differences were significant only in males (p=0.004 and p=0.001, respectively). In contrast, significant differences in basic morphology of the articular surface (Table 2c) were evident between different age groups only in females (p=0.010).

The analysis of other features (relief, porosity, and shape of the articular surface, and presence of ossific nodule) showed no relationship (p>0.05) to age groups neither within the whole sample, nor males and females separately (Tables 2c and 2d).

The applied Cohen's Kappa test estimated interobserver agreement in the range from fair to almost perfect concerning observed macroscopic features. Thus, the fair agreement was measured only in examination of presence of ossific nodule (Kappa=0.367, p<0.001). The moderate level was calculated the most frequently and it was recorded in analyses of following features: basic morphology (Kappa=0.510, $p<0.001$) and relief of the articular surface (Kappa=0.445, $p<0.001$), morphology of the margin (Kappa=0.422, $p<0.001$) and morphology of the notch for the first rib (Kappa=0.559, $p<0.001$). The substantial agreement was present in observations of shape of the articular surface (Kappa=0.700, $p<0.001$). The almost perfect agreement was estimated in analyses of epiphyseal union progress (Kappa=0.851, $p<0.001$) and porosity of the articular surface (Kappa=0.877, $p<0.001$).

Table 2a. Distribution of analysed morphological changes of articular surface by the age category (PART I).

AGE CATEGORIES	**MORPHOLOGY OF THE NOTCH FOR THE FIRST RIB**																							
	BONE OVERGROWTHS						**WITHOUT OUTSTANDING MARGINS**						**PRESENCE OF OUTSTANDING MARGINS**						**LIPPING**					
	M		F		**M & F**		M		F		**M & F**		M		F		**M & F**		M		F		**M & F**	
	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**
25 AND YOUNGER † *	0	0	0	0	**0**	**0**	5	12.8	2	8.0	**7**	**77.8**	2	5.1	0	0	**2**	**22.2**	0	0	0	0	**0**	**0**
26 – 35	0	0	0	0	**0**	**0**	8	20.5	1	4.0	**9**	**60.0**	3	7.7	3	12.0	**6**	**40.0**	0	0	0	0	**0**	**0**
36 – 45	0	0	0	0	**0**	**0**	4	10.3	3	12.0	**7**	**63.6**	1	2.6	2	8.0	**3**	**27.3**	0	0	1	4.0	**1**	**9.1**
46 – 55	0	0	0	0	**0**	**0**	7	17.9	1	4.0	**8**	**61.5**	0	0	4	16.0	**4**	**30.8**	0	0	1	4.0	**1**	**7.7**
56 AND OLDER	0	0	0	0	**0**	**0**	2	5.1	0	0	**2**	**12.5**	2	5.1	4	16.0	**6**	**37.5**	5	12.8	3	12.0	**8**	**50.0**
TOTAL	0	0	0	0	**0**	**0**	26	66.7	7	28.0	**33**	**51.6**	8	20.5	13	52.0	**21**	**32.8**	5	12.8	5	20.0	**10**	**15.6**

* The both individuals with non-union epiphyseal stage were excluded from further morphological observations.

† One individual was excluded from analyses of morphology of the notch for the first rib due to damaged medial clavicular end.

"M" stands for Male while "F" stands for Female.

Table 2b. Distribution of analysed morphological changes of articular surface by the age category (PART II).

AGE CATEGORIES	MORPHOLOGY OF THE MARGIN																							
	OBTUSE						**SHARP**						**LIPPING**						**EXOSTOSES**					
	M		F		**M & F**		M		F		**M & F**		M		F		**M & F**		M		F		**M & F**	
	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**
25 AND YOUNGER *	0	0	2	9.1	**2**	**20.0**	8	20.0	0	0	**8**	**80.0**	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**
26 – 35 **	1	2.5	2	9.1	**3**	**21.4**	10	25.0	1	4.5	**11**	**78.6**	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**
36 – 45	2	5.0	3	13.6	**5**	**45.5**	3	7.5	3	13.6	**6**	**54.5**	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**
46 – 55	4	10.0	5	22.7	**9**	**69.2**	3	7.5	0	0	**3**	**23.1**	0	0	0	0	**0**	**0**	0	0	1	4.5	**1**	**7.7**
56 AND OLDER ***	8	20.0	5	22.7	**13**	**92.9**	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**	1	2.5	0	0	**1**	**7.1**
TOTAL	15	37.5	17	77.3	**32**	**51.6**	24	60.0	4	18.2	**28**	**45.2**	0	0	0	0	**0**	**0**	1	2.5	1	4.5	**2**	**3.2**

*   The both individuals with non-union epiphyseal stage were excluded from further morphological observations.

**   One individual was excluded from analyses of the margin morphology due to damaged medial clavicular end.

*** Two individuals were excluded from analyses of the margin morphology due to damaged medial clavicular end.

“M” stands for Male while “F” stands for Female.

Table 2c. Distribution of analysed morphological changes of articular surface by the age category (PART III).

AGE CATEGORIES	**BASIC MORPHOLOGY**																		**SHAPE**																	
	CONVEX						**PLANE**						**CONCAVE**						**TRIANGULAR**						**ROUND**						**OVAL**					
	M		F		**M & F**		M		F		**M & F**		M		F		**M & F**		M		F		**M & F**		M		F		**M & F**		M		F		**M & F**	
	n	%	N	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**
25 AND YOUNGER *	0	0	2	8.0	**2**	**20.0**	4	10.0	0	0	**4**	**40.0**	4	10.0	0	0	**4**	**40.0**	2	5.0	0	0	**2**	**20.0**	1	2.5	0	0	**1**	**10.0**	5	12.5	2	8.7	**7**	**70.0**
26 – 35 **	0	0	0	0	**0**	**0**	2	5.0	4	16.0	**6**	**40.0**	9	22.5	0	0	**9**	**60.0**	6	15.0	1	4.3	**7**	**50.0**	2	5.0	2	8.7	**4**	**28.6**	3	7.5	0	0	**3**	**21.4**
36 – 45	0	0	0	0	**0**	**0**	0	0	5	20.0	**5**	**45.5**	5	12.5	1	4.0	**6**	**54.5**	2	5.0	1	4.3	**3**	**27.3**	1	2.5	2	8.7	**3**	**27.3**	2	5.0	3	13.0	**5**	**45.5**
46 – 55	0	0	1	4.0	**1**	**7.7**	4	10.0	5	20.0	**9**	**69.2**	3	7.5	0	0	**3**	**23.1**	3	7.5	3	13.0	**6**	**46.2**	1	2.5	0	0	**1**	**7.7**	3	7.5	3	13.0	**6**	**46.2**
56 AND OLDER **	0	0	0	0	**0**	**0**	4	10.0	5	20.0	**9**	**56.3**	5	12.5	2	8.0	**7**	**43.8**	3	7.5	1	4.3	**4**	**26.7**	3	7.5	2	8.7	**5**	**33.3**	3	7.5	3	13.0	**6**	**40.0**
TOTAL	0	0	3	12.0	**3**	**4.6**	14	35.0	19	76.0	**33**	**50.8**	26	65.0	3	12.0	**29**	**44.6**	16	40.0	6	26.1	**22**	**34.9**	8	20.0	6	26.1	**14**	**22.2**	16	40.0	11	47.8	**27**	**42.9**

*   The both individuals with non-union epiphyseal stage were excluded from further morphological observations.

** One individual was excluded from shape analysis due to damaged medial clavicular end.

“M” stands for Male while “F” stands for Female.

Table 2d. Distribution of analysed morphological changes of articular surface by the age category (PART IV).

AGE CATEGORIES	RELIEF												POROSITY												OSSIFIC NODULE											
	SMOOTH						**RUGGED**						**PRESENT**						**NOT PRESENT**						**PRESENT**						**NOT PRESENT**					
	M		F		**M & F**		M		F		**M & F**		M		F		**M & F**		M		F		**M & F**		M		F		**M & F**		M		F		**M & F**	
	n	%	N	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**	n	%	n	%	**n**	**%**
25 AND YOUNGER *	7	17.5	2	8.0	**9**	**90.0**	1	2.5	0	0	**1**	**10.0**	0	0	0	0	**0**	**0**	8	20.0	2	8.0	**10**	**100**	0	0	0	0	**0**	**0**	8	20.0	2	8.0	**10**	**100**
26 – 35	6	15.0	4	16.0	**10**	**66.7**	5	12.5	0	0	**5**	**33.3**	3	7.5	0	0	**3**	**20.0**	8	20.0	4	16.0	**12**	**80.0**	1	2.5	0	0	**1**	**6.7**	10	25.0	4	16.0	**14**	**93.3**
36 – 45	5	12.5	5	20.0	**10**	**90.9**	0	0	1	4.0	**1**	**9.1**	1	2.5	0	0	**1**	**9.1**	4	10.0	6	24.0	**10**	**90.9**	1	2.5	0	0	**1**	**9.1**	4	10.0	6	24.0	**10**	**90.9**
46 – 55	5	12.5	6	24.0	**11**	**84.6**	2	5.0	0	0	**2**	**15.4**	2	5.0	1	4.0	**3**	**23.1**	5	12.5	5	20.0	**10**	**76.9**	0	0	0	0	**0**	**0**	7	17.5	6	24.0	**13**	**100**
56 AND OLDER	5	12.5	6	24.0	**11**	**68.8**	4	10.0	1	4.0	**5**	**31.3**	4	10.0	4	16.0	**8**	**50.0**	5	12.5	3	12.0	**8**	**50.0**	2	5.0	1	4.0	**3**	**18.8**	7	17.5	6	24.0	**13**	**81.3**
TOTAL	28	70.0	23	92.0	**51**	**78.5**	12	30.0	2	8.0	**14**	**21.5**	10	25.0	5	20.0	**15**	**23.1**	30	75.0	20	80.0	**50**	**76.9**	4	10.0	1	4.0	**5**	**7.7**	36	90.0	24	96.0	**60**	**92.3**

* The both individuals with non-union epiphyseal stage were excluded from further morphological observations.

“M” stands for Male while “F” stands for Female.

Laboratory Study – Histology

Cortical width (Table 3) did not show any correlation with age either in males (R^2=0.060, p=0.468) or females (R^2=0, p=0.949) (Table 6). Nevertheless, cortical width maintained higher values in men than in women (p=0.004).

Table 3. The average values of recorded cortical width and minimum trabecular width (presented in µm).

		Min	Max	Mean	SE
Female	Cortical width	40.8	1260.9	383.5	11.1
	Min Trabecular Width	57.0	363.7	134.5	2.7
Male	Cortical width	90.8	1709.5	643.1	18.0
	Min Trabecular Width	32.0	639.9	150.7	3.8
Whole Sample	Cortical width	40.8	1709.5	513.3	11.7
	Min Trabecular Width	32.0	639.9	142.6	2.3

Minimum trabecular width differed (p=0.053) between the sexes (males: 150.7µm, females: 134.5µm) (Table 3). The conducted analyses could not determine correlation between minimum trabecular width and age in female part of the sample (R^2=0.028, p=0.622) (Table 6). However, the correlation was ascertainable in the whole sample (R^2=0.179, SE=18.41, p=0.050), due to significant age-related trabecular thinning in male part of the sample (R^2=0.514, SE=14.62, p=0.013). The obtained relations (Table 6) allowed implementation of following equations:

- for the whole sample:

$$Tb.Wi = 163.446 - 0.418 \cdot Age$$

- for the male part of the sample:

$$Tb.Wi = 185.773 - 0.708 \cdot Age$$

Trabecular bone volume fraction was 29% on average in males and 26% in females (Table 4 and 5), however without statistically significant intersex differences (p=0.438).

Table 4. The average values of recorded trabecular bone area (presented in µm^2).

	Min	Max	Mean	SE
Female	619248.0	2032784.0	1290040.7	60021.0
Male	724992.0	2245664.0	1395039.0	74381.5
Whole Sample	619248.0	2245664.0	1342539.9	47864.9

Table 5. The average values of derived bone-tissue area ratio.

	Min	Max	Mean	SE
Female	0.13	0.41	0.26	0.01
Male	0.15	0.46	0.29	0.02
Whole Sample	0.13	0.46	0.28	0.01

Our analysis revealed significant correlation between bone volume fraction and individual's age in the whole sample (R^2=0.259, SE=0.06, p=0.016) as well as in male part of the sample (R^2=0.492, SE=0.06, p=0.016). Conversely, such correlation could not be determined in female part of the sample (R^2=0.085, p=0.383). Thus, the relations (Table 6) between age and bone volume fraction was given in the equations:

- for the whole sample:

$$Cn.B.Ar/T.Ar = 0.359 - 0.002 \cdot Age$$

- for the male part of the sample:

$$Cn.B.Ar/T.Ar = 0.416 - 0.003 \cdot Age$$

Table 6. The results of conducted univariate analyses of obtained histomorphometric data in relation to individual's age.

Variables	SEX	b	SE of the Estimate	t	p	β	95% Confidence Interval for B	
							Lower Bound	Upper Bound
Ct.Wi	M	-2.840	239.103	-0.757	0.468	-0.245	-11.324	5.644
Ct.Wi	F	-0.131	130.940	-0.066	0.949	-0.022	-4.609	4.347
Tb.Wi	W	-0.418	18.408	-2.085	0.050	-0.423	-0.836	0
Tb.Wi	M	-0.708	14.619	-3.087	0.013	-0.717	-1.227	-0.189
Tb.Wi	F	-0.135	17.553	-0.510	0.622	-0.168	-0.736	0.465
Cn.B.Ar/T.Ar	W	-0.002	0.059	-2.642	0.016	-0.509	-0.003	0
Cn.B.Ar/T.Ar	M	-0.003	0.057	-2.950	0.016	-0.701	-0.005	-0.001
Cn.B.Ar/T.Ar	F	-0.001	0.059	-0.917	0.383	-0.292	-0.003	0.001

"W" stands for whole sample while "F" stands for female and "M" for male.

Clinical Study – Clavicles

According to the applied Wilcoxon test side differentiation of medial clavicular epiphyseal cartilage ossification status was detected neither in the whole sample ($p=0.622$) nor in separately analysed male- ($p=0.564$) and female-sample-part ($p=1.000$). In addition, although females showed slight tendency towards higher epiphyseal union stages, the observed sexual dimorphism was not statistically significant (OR=0.86, $p=0.498$). Therefore, the both features were disregarded from further analyses (Table 7). The first stage was recorded predominantly in 15-year-old individuals (63.6%) and could be seen until the age of 17. However, the second stage almost entirely overlapped with the first, being present in the range between 15 and 18 years. In addition, the second stage was mainly recorded (70.0%) among 17 and 18-year-old individuals. Considering the third stage, the latest detection was at the age of 25, and its radiological characteristics could be traced until the age of 17 with only one recording at the age of 15 (2.1%). The fourth stage of epiphyseal fusion was observed in the age range from 19 to 30 years, with three quarters (75.5%) of recordings in the range between 20 and 25 years of age. Finally, the last – fifth stage thoroughly overlapped with the fourth showing the earliest detection at the age of 19, and extended mainly (98.8%) from the age of 22 until 35 years of age. The conducted statistical analysis confirmed significant correlation between the stage of epiphyseal fusion and age ($p<0.001$), revealing the low level of stage discrimination only between the first two stages ($p>0.05$) while mutual distinction between all other stages were statistically significant ($p<0.05$).

Table 7. Distribution of progress of medial clavicular epiphyseal cartilage ossification status by individual's age in the whole sample.

STAGE	FREQUENCY		INDIVIDUAL'S AGE				
	n	%	Min	Max	Mean	Mod	SD
1	22	7.14	15	17	15.5	15	0.7
2	20	6.49	15	18	16.9	17	1.1
3	48	15.58	15	25	19.7	20	2.0
4	49	15.91	19	30	23.8	25	2.8
5	169	54.87	19	35	28.3	24	4.0

The sex variability also could not be detected among acquired values of radiodensity of sternal epiphyseal-metaphyseal region (p=0.499 for right side, and p=0.182 for left side). However, it differed among sides in the whole sample (p=0.036) and separately analysed female part of the sample (p=0.015). The average values of 276.78 HU for the right side and 269.16 HU for the left side were observed in the whole sample (Table 8), while average values of 271.46 HU for the right and 258.19 HU for the left side were recorded in the female-sample-part (Table 8).

Table 8. The average values of recorded sternal metaphyses radiodensity (presented in Hounsfield unit – HU).

		Min	Max	Mean	SE
Female	Right	108.50	430.50	271.46	9.87
	Left	72.10	411.80	258.18	9.86
	Right & Left	**72.10**	**430.50**	**264.76**	**6.97**
Male	Right	120.90	458.70	279.86	7.50
	Left	103.70	492.90	275.61	8.10
	Right & Left	**103.70**	**492.90**	**277.73**	**5.51**
Whole Sample	Right	108.50	458.70	276.78	5.96
	Left	72.10	492.90	269.16	6.29
	Right & Left	**72.10**	**492.90**	**272.96**	**4.33**

Nevertheless, side variability could not be noticed only in separately analysed male-sample-part (p=0.364). Further analyses (Table 13) revealed significant correlation between individual's age and bilaterally observed radiodensity of sternal epiphyseal-metaphyseal region in the whole sample (R^2=0.446, SE=55.08, p<0.001 for right side; R^2=0.389, SE=61.25, p<0.001 for left side). Furthermore, additionally conducted analyses without side differentiation in the male-sample-part confirmed previously stated statistical significance (R^2=0.399; SE=59.66; p<0.001). The obtained relations allowed implementation of the following equations:

- for the whole sample, right clavicle:

$$Ep.Dn = 490.059 - 8.655 \cdot Age$$

- for the whole sample, left clavicle:

$$Ep.Dn = 479.954 - 8.565 \cdot Age$$

- additional equation for the male part of the sample:

$$Ep.Dn = 493.032 - 8.879 \cdot Age$$

The analyses of obtained thickness of the anterior (Table 9) and posterior (Table 10) cortex allowed side discrimination in the whole sample, as well as in the separately analysed male and female parts of the sample ($p<0.05$). Furthermore, the results revealed sex difference only in recorded thickness of anterior cortex of right side clavicles ($p=0.042$).

Table 9. The average values of recorded thickness of the anterior cortex (presented in mm).

		Min	Max	Mean	SE
Female	Right	0.13	0.45	0.23	0.008
	Left	0.15	0.37	0.25	0.006
	Right & Left	**0.13**	**0.45**	**0.24**	**0.005**
Male	Right	0.10	0.36	0.21	0.005
	Left	0.14	0.43	0.24	0.006
	Right & Left	**0.10**	**0.43**	**0.23**	**0.004**
Whole Sample	Right	0.10	0.45	0.22	0.005
	Left	0.14	0.43	0.24	0.004
	Right & Left	**0.10**	**0.45**	**0.23**	**0.003**

Table 10. The average values of recorded thickness of the posterior cortex (presented in mm).

		Min	Max	Mean	SE
Female	Right	0.17	0.35	0.25	0.005
	Left	0.18	0.35	0.27	0.005
	Right & Left	**0.17**	**0.35**	**0.26**	**0.004**
Male	Right	0.14	0.45	0.25	0.005
	Left	0.13	0.47	0.28	0.005
	Right & Left	**0.13**	**0.47**	**0.26**	**0.004**
Whole Sample	Right	0.14	0.45	0.25	0.004
	Left	0.13	0.47	0.27	0.004
	Right & Left	**0.13**	**0.47**	**0.26**	**0.003**

The observed anterior cortex thickness in the male- and female parts of the sample did not expressed age dependence ($R^2=0.032$, $p=0.081$ and $R^2=0.008$, $p=0.379$, for the right and left side, respectively in the male-, while $R^2=0$, $p=0.954$ and $R^2=0.026$, $p=0.229$ in the female-sample-part) (Table 13). Similarly, the dependence of the posterior cortex thickness on the individual's age was not found in either side (R^2=0.001, p=0.655 for the right side, and R^2=0.004, p=0.428 for the left side) (Table 13). Finally, the calculated anterior to posterior cortical thickness ratio although differed among sexes (p=0.003) did not express statistically significant side differences (p>0.05). The analyses (Table 13) detected correlation between the calculated anterior to posterior cortical thickness ratio and individual's age among males (R^2=0.022, SE=0.19, p=0.041). Thus, the determined relation was given in the equation:

$$Ct.Th_{ant}/Ct.Th_{post} = 0.993 - 0.005 \cdot Age$$

The conducted tests could not assign sexual equality (p<0.001) to analysed diameter of the medullar canal (Table 11), diameter of the clavicular shaft (Table 12), and calculated medullar to shaft diameter ratio.

Table 11. The average values of recorded diameter of medullar canal (presented in cm).

		Min	Max	Mean	SE
Female	Right	0.37	1.15	0.69	0.021
	Left	0.39	1.07	0.64	0.020
	Right & Left	**0.37**	**1.15**	**0.66**	**0.015**
Male	Right	0.43	1.52	0.94	0.022
	Left	0.55	1.32	0.88	0.018
	Right & Left	**0.43**	**1.52**	**0.91**	**0.014**
Whole Sample	Right	0.37	1.52	0.85	0.019
	Left	0.39	1.32	0.79	0.017
	Right & Left	**0.37**	**1.52**	**0.82**	**0.013**

Table 12. The average values of recorded diameter of clavicular shaft (presented in cm).

		Min	Max	Mean	SE
Female	Right	0.77	1.53	1.16	0.020
	Left	0.93	1.62	1.17	0.020
	Right & Left	**0.77**	**1.62**	**1.17**	**0.008**
Male	Right	1.00	1.91	1.40	0.020
	Left	0.98	1.85	1.41	0.018
	Right & Left	**0.98**	**1.91**	**1.41**	**0.013**
Whole Sample	Right	0.77	1.91	1.31	0.017
	Left	0.93	1.85	1.32	0.016
	Right & Left	**0.77**	**1.91**	**1.32**	**0.012**

Considering both male and female subsamples, as well as the whole sample, side differentiation was observed in the diameter of the medullar canal ($p<0.001$) and calculated medullar to shaft diameter ratio ($p<0.05$), while recorded diameter of clavicular shaft did not express statistically significant signs of bilateralism ($p=0.566$ in whole sample; $p=0.786$ in males; $p=0.550$ in females). Subsequently conducted analyses revealed significant correlation in the male part of the sample between individual's age and the recorded diameter of clavicular shaft ($R^2=0.064$, SE=0.18, $p<0.001$) as well as bilaterally observed diameters of the medullar canal ($R^2=0.056$, SE=0.21, $p=0.020$ for the right side, and $R^2=0.078$, SE=0.17, $p=0.006$ for the left side). Therefore, the analyses of clavicular shaft (Table 13) permitted the creation of several more age-related equations for the male part of the sample:

- for diameter of clavicular shaft, male sample, without side differentiation:

$$Dp.Wi = 1.198 + 0.009 \cdot Age$$

- for diameter of the medullar canal, male sample, right clavicle:

$$Ca.Wi = 0.721 + 0.009 \cdot Age$$

- for diameter of the medullar canal, male sample, left clavicle:

$$Ca.Wi = 0.657 + 0.009 \cdot Age$$

Nevertheless, the calculated medullar to shaft diameter ratio did not depend on age either in the whole sample or in separately analysed male and female parts of the sample, concerning both sides ($p>0.05$) (Table 13). Finally, the analyses of the same kind conducted in the female-sample-part did not confirm any correlation between age and recorded data ($p>0.05$) (Table 13).

Table 13. The results of conducted univariate analyses of obtained clavicular CT data in relation to individual's age.

Variables	SEX	Body Side	b	SE of the Estimate	t	p	β	95% Confidence Interval for B	
								Lower Bound	Upper Bound
Ep.Dn	W	R	-8.655	55.080	-11.022	0.000	-0.668	-10.207	-7.104
Ep.Dn	W	L	-8.565	61.253	-9.829	0.000	-0.623	-10.287	-6.844
Ep.Dn	M	B	-8.879	59.656	-11.281	0.000	-0.631	-10.432	-7.327
Ep.Dn	F	R	-8.545	53.366	-7.172	0.000	-0.698	-10.934	-6.157
Ep.Dn	F	L	-7.775	58.381	-6.007	0.000	-0.629	-10.370	-5.181
$Ct.Th_{ant}$	M	R	-0.002	0.052	-1.765	0.081	-0.179	-0.004	0
$Ct.Th_{ant}$	M	L	-0.001	0.059	-0.883	0.379	-0.091	-0.003	0.001
$Ct.Th_{ant}$	F	R	$7.71E^{-005}$	0.060	0.058	0.954	0.008	-0.003	0.003
$Ct.Th_{ant}$	F	L	-0.001	0.047	-1.216	0.229	-0.162	-0.003	0.001
$Ct.Th_{post}$	W	R	0	0.047	0.448	0.655	0.036	-0.001	0.002
$Ct.Th_{post}$	W	L	-0.001	0.046	-0.794	0.428	-0.065	-0.002	0.001
$Ct.Th_{ant}/Ct.Th_{post}$	M	B	-0.005	0.194	-2.055	0.041	-0.147	-0.010	0
$Ct.Th_{ant}/Ct.Th_{post}$	F	B	-0.002	0.197	-0.777	0.439	-0.073	-0.009	0.004
Ca.Wi	M	R	0.009	0.209	2.359	0.020	0.236	0.001	0.017
Ca.Wi	M	L	0.009	0.174	2.816	0.006	0.279	0.003	0.016
Ca.Wi	F	R	0.004	0.156	1.243	0.219	0.165	-0.003	0.011
Ca.Wi	F	L	0.002	0.155	0.494	0.623	0.067	-0.005	0.009
Dp.Wi	M	W	0.009	0.179	3.608	0.000	0.253	0.004	0.013
Dp.Wi	F	W	0.003	0.145	1.212	0.228	0.114	-0.002	0.007
Ca.Wi/Dp.Wi	M	R	0.003	0.081	1.942	0.055	0.196	0	0.006
Ca.Wi/Dp.Wi	M	L	0.002	0.074	1.473	0.144	0.150	-0.001	0.005
Ca.Wi/Dp.Wi	F	R	0.002	0.083	1.024	0.310	0.137	-0.002	0.006
Ca.Wi/Dp.Wi	F	L	0.001	0.081	0.468	0.642	0.063	-0.003	0.004

"W" stands for whole sample while "F" stands for female and "M" for male.

"B" stands for both sides while "R" stands for right side and "L" for left side.

Overall, due to image artefacts clavicular analyses excluded one right sided medial clavicle from radiodensity measurements while one per both sides were excluded from all other analyses except of estimation of progress of medial clavicular epiphyseal cartilage ossification status which encompassed the maximal number of 308 clavicles.

Considering epiphyseal union stages, the applied Cohen's Kappa test determined substantial agreement between two observers (right side: Kappa=0.708, $p<0.001$; left side: Kappa=0.678, $p<0.001$). On the other hand, in order to assess reproducibility of quantitative measurements made by different observers the Interclass correlation coefficient was applied. Therefore, the calculated values demonstrated excellent agreement concerning radiodensity of the sternal epiphyseal-metaphyseal region (right side: ICC=0.918, $p<0.001$; left side: ICC=0.914, $p<0.001$), enlargement of medullar canal (right side: ICC=0.928, $p<0.001$; left side: ICC=0.926, $p<0.001$), and diameter of clavicular shaft (right side: ICC=0.950, $p<0.001$; left side: ICC=0.957, $p<0.001$). However, the interobserver agreement was not so well but still optimal regarding thickness of the anterior (right side: ICC=0.708, $p<0.001$; left side: ICC=0.751, $p<0.001$) and posterior cortex (right side: ICC=0.767, $p<0.001$; left side: ICC=0.725, $p<0.001$).

Clinical Study – The First Costal Cartilage

Considering the analysed features of the first costal cartilage, the results revealed side ($p>0.05$) and sex ($p>0.05$) uniformity among selected 154 individuals.

The measuring of costal cartilage radiodensity (Table 14) excluded two cartilages (one per both sides) due to image artefacts, and therefore was performed on total of 306 cartilages providing average value of 130.39 HU.

Table 14. The average values of recorded radiodensity of the first costal cartilage and calculated relation of projection's length to distance between costal face and sternum in the whole sample.

	Min	Max	Mean	SE
Radiodensity of the First Costal Cartilage (presented in HU)	40.70	391.50	130.39	3.57
Relation of Projection's Length to Distance between Costal Face and Sternum	0.12	1.00	0.66	0.01

The applied statistics detected significant correlation between individual's age and cartilage radiodensity (R^2=0.307, SE=52.21, p<0.001) which was even more reliable after the process of data normalisation (R^2=0.378, SE=0.35, p<0.001). The estimated relations (Table 16) were presented in the following equations:

- relation between the first cartilage radiodensity and individual's age:

$$FC.Dn = 6.101 \cdot Age - 19.911$$

- relation between the first cartilage radiodensity and individual's age, after data normalisation:

$$\ln FC.Dn = 3.603 + 0.047 \cdot Age$$

Most frequently costal cartilage was attributed with one calcified linear projection (47.1%), while two beaks were recorded in 9.7%. Considering both, single OCP and two OCPs, the first detection of projections was made in a 19-year-old-individual and could be followed until the 35 years of age. The non-linear appearances of calcifications were noted in only 4.5% of all examined cartilages, always after the age of 23. The absence of linear projections was noted in 38.6% and could be seen in almost all ages from 15 to 34 years of age, with almost half (48.7%) of all observations in the age range between 15 and 18 years. The conducted analyses estimated statistically significant correlation between individual's age and calculated stages of the bony changes of the anterior and posterior margins of the costal face (p<0.001) (Table 15). However, results revealed satisfying distinction only between the first and all subsequent stages (p<0.001), while the mutual difference between the second, third and fourth stage could not be identified (p>0.05). Therefore, the author analysed relation of projection's length to distance between the costal face and sternum (Table 14), and calculated values tested in correlation to individual's age. The determined correlation (R^2=0.065, SE=0.18, p=0.001) (Table 16) was presented by the following equation:

$$OCP.Le/CS.Le = 0.360 + 0.011 \cdot Age$$

Table 15. Distribution of stages of the bony changes of anterior and posterior margin of costal face in the whole sample.

STAGE	FREQUENCY		INDIVIDUAL'S AGE			
	n	%	Min	Max	Mean	SD
1	119	38.64	15	34	20.08	4.37
2	34	11.04	19	34	25.59	4.39
3	141	45.78	19	35	27.75	4.37
4	14	4.55	23	35	29.07	3.71

Table 16. The results of conducted univariate analyses of obtained cartilage CT data in relation to individual's age.

Variables	SEX	Body Side	b	SE of the Estimate	t	p	β	95% Confidence Interval for B	
								Lower Bound	Upper Bound
FC.Dn	W	B	6.101	52.210	11.600	0.000	0.554	5.066	6.254
ln FC.Dn	W	B	0.047	0.346	13.604	0.000	0.615	0.041	0.054
OCP.Le/CS.Le	W	B	0.011	0.183	3.474	0.001	0.255	0.005	0.017

"W" stands for the whole sample.

"B" stands for both body sides.

The analyses of interobserver reliability revealed almost perfect agreement concerning calculated stages of the morphological appearances of the first costal cartilage (right side: Kappa=0.888, p<0.001; left side: Kappa=0.887, p<0.001), as well as for the number and type of the bony changes of anterior and posterior margins of the costal face (right side: Kappa=0.906, p<0.001; left side: Kappa=0.815, p<0.001). Considering acquired quantitative measurements, the applied Interclass correlation coefficient detected excellent agreement regarding obtained radiodensity of the costal cartilage (right side: ICC=0.982, p<0.001; left side: ICC=0.984, p<0.001), measured beak length at the left side (ICC=0.962, p<0.001), as well as measured distance between costal face and sternum also at the left side (ICC=0.890, p<0.001). Unexpectedly, the interobserver agreement was not satisfactory concerning measurements acquired at the right side such as beak length (ICC=0.469, p=0.002), and distance between costal face and sternum (ICC=0.182, p=0.181). However, subsequently conducted analyses of the relation of projection's length to distance between costal face and sternum revealed excellent agreement between observers at both sides (right side: ICC=0.946, p<0.001; left side: ICC=0.952, p<0.001).

Clinical Study – Mutual Analyses of Clavicles and the First Costal Cartilage

Subsequently conducted analyses of mutual relation of observed clavicular and the first costal cartilage features to individual's age encompassed only those features that previously independently analysed already expressed statistically significant correlation to age without intersex variability. The side differentiation was disregarded in further analyses.

Although separately analysed stages of the ossification status of medial clavicular epiphyseal cartilage and stages of the bony changes of the first costal cartilage were statistically significantly influenced by age, when their mutual relation was tested to individual's age the results revealed that the observed interaction did not correlate with age (p=0.564).

Finally, in order to resolve age classification problem, the author tested age in relation to acquired values of radiodensity of sternal epiphyseal-metaphyseal region and calculated relation of OCP's length to distance between costal face and sternum (R^2=0.372, SE=3.545, p<0.05). Similar results were attained after testing age in relation to three acquired variables: radiodensity of sternal epiphyseal-metaphyseal region, radiodensity of the first costal cartilage, and calculated relation of OCP's length to distance between costal face and sternum (R^2=0.398, SE=3.480, p<0.05). However, statistical results excluded calculated relation of OCP's length to distance between costal face and sternum from this equation (p=0.579). Nevertheless, the analyses suggested individual usage of radiodensity of sternal epiphyseal-metaphyseal region as more precise predictor of age (R^2=0.414, p<0.001). Furthermore, the mutual relation of radiodensity of sternal epiphyseal-metaphyseal region and radiodensity of the first costal cartilage allowed creation of more reliable equation (R^2=0.557; SE=3.80; $p<0.001$), which was even more pronounced after the process of data normalization (R^2=0.588; SE=3.66; $p<0.05$) (Table 17). Thus, two equations were created:

$$Age = 0.035 \cdot FC.Dn - 0.039 \cdot Ep.Dn + 30.831$$

$$Age = 5.695 \cdot \ln FC.Dn - 0.037 \cdot Ep.Dn + 7.534$$

Table 17. The results of conducted univariate and multivariate analyses of age classification problem in relation to obtained clavicular and cartilage CT data in the whole sample without side differentiation.

Variables		Univariate Analyses							Multivariate Analyses						
		b	SE of the Estimate	t	p	β	95% Confidence Interval for B		b	SE of the Estimate	t	p	β	95% Confidence Interval for B	
							Lower Bound	Upper Bound						Lower Bound	Upper Bound
Ep.Dn	1st equation	-0.048	4.352	-14.687	0.000	-0.644	-0.055	-0.042	-0.039	3.797	-13.066	0.000	-0.526	-0.045	-0.034
	2nd equation								-0.037	3.658	-12.434	0.000	-0.491	-0.043	-0.031
FC.Dn		0.050	4.740	11.600	0.000	0.554	0.042	0.059	0.035	3.797	9.640	0.000	0.388	0.028	0.042
ln FC.Dn		7.982	4.489	13.604	0.000	0.615	6.828	9.137	5.695	3.659	11.114	0.000	0.439	4.686	6.703

Discussion

The Review of Epiphyseal Union Timing

Although the previously conducted morphological analyses of the clavicular articular surface allowed differentiation of several age-related features, some inconsistencies remained mostly due to differences in methodology. Following McKern and Stewart's publication (7), Henle was the first to report the time of the medial epiphyses union in the year of 1871. The published age of 18 years was afterwards also reported by Dwight in 1911. However, the other authors who also took activity in the early years of the twentieth century reported different results. Thus, Kraus (1909) published the epiphyseal union at the age of 20-21. Afterwards, in the following decade, several authors reported epiphyseal union at 25 years of age, involving Poirier (1911), Dixon (1912), Bryce (1915), Lewis (1918), Terry (1921), and Thompson (1921), while Testut (1921) defined age range from 22 to 25 years.

The literature (7, 8) outlined the Stevenson's publication in the year of 1924 (6). The survey was conducted for the first time on a sample derived from American population which encompassed 110 complete skeletons from Western Reserve collection with known age at death in range from 15 to 28 years. The newly developed four-phase scoring system was applied at all examined epiphyseal regions. The methodology included following stages: no union, beginning union, recent union, and complete union. Especially outlined was the third stage that was according to the author the most difficult for distinction as the presence of the fine line of demarcation could easily be replaced for "epiphyseal scar" which was characteristic of the following stage. Concerning medial clavicles, the beginning of epiphyseal union was reported at the age of 22, and completed union after the age of 28. In addition, sexual dimorphism was not detected, however, the small number of encompassed females (only 20) was acknowledged as serious study limitation.

In the following years, clavicles from the Western Reserve collection were once again encompassed, this time analysed by Todd and D'Errico (1928) (7-9). The comprehensive study of medial clavicles encompassed 165 individuals with age at death in range between 17 and 29 years. The new four-phase scoring system was developed and it slightly differed from the previous, involving following stages: no union, beginning union, recent union with a scar, and complete union without traces of union. The conducted analyses revealed beginning of union at the age of 21, complete union since 25 years of age with possibility of the recent union signs until the age of 29. Overall, the sexual and ethnical variability was not observed.

Afterwards, McKern and Stewart extended the Todd and D'Errico's four-phase scoring system to five-phase, introducing in a middle phase known as "active union" phase (7). The presented results (Table 18) marked 25 years of age as the latest age of non union, while the earliest age of complete union was set at 23 years. The authors for the first time noted bilateralism but without statistical significance. Unfortunately, this comprehensive study was comprised of only males and therefore scientific public was left scarce of any intersex analyses.

Finally in 1985, contemporary population of both sexes were analysed by Webb and Suchey (9) (Table 18). The survey encompassed American population. The osteological sample was derived from autopsies and subsequently scored by four-phase system: non-union without separate epiphyses, non-union with separate epiphysis, partial union, and complete union. The non-union phases could be traced until the age of 25 in males, and 23 in females, while the earliest age of complete union was marked at 21 and 20 years of age in males and females, respectively. In addition, although concluded similar epiphyseal timing in both sexes, the authors noted variability in female sample with broader age ranges compared to males.

At the turn of the twentieth century, Black and Scheuer (5) published comparative analyses of skeletal material from archaeological Spitalfields' collection dated at the eighteenth and nineteenth century, and contemporary sample of St Bride, St. Barnabus, and Portuguese Museum Bocage's collections. The methodology was based on newly developed five-phase system which involved following stages: the first phase was characterised with clear ridge and furrow systems of the metaphyseal surface; in the second phase ridges and furrows were still present but less distinctive; the main feature of the third phase was commencement of epiphyseal flake fusion to the metaphyseal surface; the expansion of epiphyseal flake across the surface was marked at the fourth phase with still visible distinction between the epiphyseal and diaphyseal surfaces; the fifth phase was featured with the complete fusion and disappearance of fusion line. The results deferred depending on the sample source. Thus, according to the results (Table 18) the first two phases which corresponded to non-union stage were recorded until the age of 21-22, the third and fourth phases which were matched to partial union were noted since 19-22 years until 27-28 years of age while the earliest signs of complete union were observed at 25-29 years.

More recently, in 2005, another comparative study was carried out. This time, Schaefer and Black (12) analysed contemporary population of different origin i.e. American and Bosnian population. The survey examined time of fusion of various skeletal epiphyses including medial clavicles. The authors applied previously presented McKern and Stewart's scoring methodology (7). The results (Table 18) revealed significant differences between samples. Therefore, the latest age of non-union among Americans was observed at 30 years of age while in Bosnian sample it was sat at 22. Concerning both samples, the signs of partial union could be seen since the age of 18 until the 30 years of age in American and 28 years of age in Bosnian sample. The complete union was recorded after the age of 23 and 21 in American and Bosnian sample, respectively. Afterwards, Schaefer and Black developed a three-phase scoring system which was presented in a new paper in 2007 (10). The study once again encompassed Bosnian population and derived results (Table 18) were similar to previously published study from 2005. Thus, the first stage i.e. non-union could be seen until 23 years of age. The signs of second stage (partial union) were recorded in the age range from 17 to 29 years while the earliest complete union which presented third stage was observed at 21 years of age.

The five-phase scoring system which was presented by McKern and Stewart's (7) and subsequently used by Schaefer and Black (12) was also applied on Indian population by Singh and Chavali in 2011 (11). The publication revealed intersex variation (Table 18) presenting signs of non union until the age of 23 and 22 in males and females, respectively. The partial union was observed form 18 to 32 years in males, and 20 to 30 years in females. The earliest age of complete union was 22 years in both sexes. The authors also noted slight discrepancy between body sides.

Table 18. Comparative overview of the epiphyseal union stage timing through different studies with applied direct inspection method.

Study group	Year	Population	Staging System	Sample Size (N)	Males (N)	Females (N)	Age Range (in years)		Latest Age of Non Union Male/Female (in years)	Earliest Age of Partial Union Male/Female (in years)	Latest Age of Partial Union Male/Female (in years)	Earliest Age of Complete Union Male/Female (in years)
							min	max				
The Current Study	2013	Serbian	3-Phase	67	42	25	20	90	22	21	65	31
Singh J. and Chavali K. (11)	2011	Indian	5-Phase	343	252	91	17	94	23/22	18/20	32/30	22
Schaefer M. and Black S. (10)	2007	Bosnian	3-Phase	258	258	0	14	30	23	17	29	21
Schaefer M. and Black S. (12)	2005	Bosnian	5-Phase	114	114	0	17	30	22	18	28	21
Schaefer M. and Black S. (12)	2005	American	5-Phase	325	325	0	17	30	30	18	30	23
Black S. and Scheuer L. (5)	1996	Spitalfieds collection	5-Phase	67	37	30	0	30	22	22	27	26
Black S. and Scheuer L. (5)	1996	St Bide's collection	5-Phase	33	22	11	0	29	21	22	28	25
Black S. and Scheuer L. (5)	1996	Portuguese collection	5-Phase	41	22	19	0	30	21	19	27	29
Webb P. and Suchey J. (9)	1985	American	4-Phase	859	605	254	11	40	25/23	17/16	30/33	21/20
McKern T. and Stewart T. (7)	1957	American	5-Phase	374	374	0	17	31	25	18	30	23

NA – Not Available.

The Question of Epiphyseal Union Timing Comparability

Heretofore, the only analyses which actually involved similar population as the current study were carried out by Schaefer and Black and encompassed Bosnian population (10, 12). The Bosnia represents the nearby country of similar economic and healthcare system as Serbia (26, 27, 35). Schaefer and Black noticed that the “non-union” stage was present until 23 years of age while “partial” and “complete” union were obtained from the age of 17 and 21, respectively (10, 12). Those findings were fairly in concordance with the results of Singh and Chavali, and McKern and Stewart who also applied direct skeletal inspection but in different populations (7, 11). The results of the present study reported “non-union” phase until 22 years of age, whereas “partial” and “complete” union were present since 21 and 31 years, respectively. Similar age ranges were recorded in historical Portuguese sample from Black and Scheuer’s study (5). The observed discrepancy between our and other macroscopic studies of the various authors (Table 18) probably arose due to the following reasons: majority of analyses encompassed only males, studies were focused just on epiphyseal union timing, and predominantly enrolled only population younger than 30 years (5, 7, 10-12, 16, 18, 25).

Furthermore, plethora of specified constraints considerably limited comparability of published studies. Thus, as the developed multiphase scoring systems favoured subjective error in direct skeletal analyses, the three-phase staging system was proposed (8, 10, 36). However, the recommended new scoring system was only applied by Schaefer and Black and in the macroscopic section of the current study.

Additional problem in age estimation based on medial clavicle was introduced with population-specific variety. Heretofore, no definitive conclusions have been drawn whether the age ranges established in a certain population could be useful in age assessment of another. Nevertheless, the differences of attained complete union in different populations were reported by Black and Scheuer as well as Schaefer and Black (5, 12). Shirley not only emphasised the importance of ethnicity and socioeconomic influence for epiphyseal maturation, but also alluded to reasonableness in applying contemporary age-related standards only to the modern population (8).

The Role of Medial Clavicular Macroscopic Morphology

Similarly to McKern and Stewart (7) who outlined pronounced irregularity of articular surface basic morphology from concave to convex as well as varieties of articular surface shape, the current study also did not manage to determine any age relevant correlation. However, the additional macroscopic analyses conducted in the present study allowed differentiation of couple more age indicators which could be easily observed by naked eye. The results suggested that signs of lipping in the region of the notch for the first rib as well as exostoses and bone overgrowths of the articular surface margin should be considered as attributes of older individuals, as this features were noticed in individuals after 44 and 53 years, respectively.

Overall, the subtle morphological changes during the process of epiphyseal union are more easily detected for a longer period of time by anatomical preparation. Therefore, wherever it is applicable as the most reliable approach was proposed macroscopic morphological examinations by Singh and Chavali (11). However, the remarkable age related changes of clavicular radiological features could be found in the previous literature (19, 32). Still, often different interpretations emphasised method-specific differences in age ranges that could be ascribed to certain features (5, 8, 11, 16, 18, 25, 37). Therefore, it was proposed strict usage of scoring systems only for sample-types for which were developed i.e. gross sample demands scoring methodology developed for direct skeletal inspection while radiographs requires radiology-based scoring system (5, 8, 11, 25, 37).

The Review of Epiphyseal Ossification Timing

Methodology impediments were introduced by disposition of particular procedure. In this manner, radiological approach can affect age estimation due to intrinsic optical and tissue superimpositions especially in PA projections but it is more prone to early detection of ossification. Therefore, the progress was to be expected with introduction of modern radiological techniques in the field of age estimation. Computed tomography was for the first time applied for this purpose in 1998 by Kreitner et al. (18). The authors presented the four-phase scoring system which was based on Webb and Suchey's methodology although it was primarily developed for direct skeletal inspection (9). Thus, the first stage was assigned in cases of non-union without traces of epiphyseal ossification; the onset of epiphyseal ossification without signs of union referred to the second stage; the third stage represented partial union; and finally fourth stage was defined as complete union. The conducted analyses did not revealed statistically significant differences between body sides or intersex variation. According to the results (Table 19), the signs of non-union could be seen until 22 years of age which was the same age of the earliest observation of complete union. The partial union was recorded in individuals aged from 16 to 26 years.

The existing CT methodology sustained the only modifications in 2005, after the publication Schulz et al. (31). Conversly to Kreitner, Schulz et al. introduced new modified scoring system which was in accordance with classification methodology for conventional radiology developed by Schmeling et al. (25). Thus, it comprised five stages: the first stage was assigned when the ossification centre was not ossified; the second stage referred to ossification centre which was ossified while epiphyseal cartilage was not; the third stage corresponded to partly ossified epiphyseal cartilage; in cases of fully ossified epiphyseal cartilage with still visible epiphyseal scar, the fourth stage was assigned; the fifth stage was represented by fully ossified epiphyseal cartilage with no longer visible epiphyseal scar. Unfortunately, the authors did not define the age boundaries of each stage providing only the earliest age of partly ossified epiphyseal cartilage and fully ossified epiphyseal cartilage (Table 19). The published data revealed intersex variation only in the second stage, while expressed bilateralism was not statistically significant. Finally, the recommended slice thickness was defined and set at 1mm.

In the following year, Schulze et al. (38) presented results of their survey (Table 19). The authors applied four-phase scoring system which was in accordance with methodology of Kreitner et al. (18). The results were fairly in agreement with both previously published studies. Once more, statistics did not detected variation between body sides or among sex.

In the last few years, several studies based on CT methodology of age estimation were published. Thus, in 2010 Kellinghaus et al. (39) applied Schmeling's methodology (25) and presented results for both sexes (Table 19). The not ossified epiphyseal cartilage was observed until the age of 20 in males and 19 in females. The partly ossified epiphyseal cartilage was seen until the 26 years of age in both sexes. Similarly to others, the earliest signs of fully ossified epiphyseal cartilage were set at 22 and 21 years of age, in males and females, respectively. The observed intersex variation was statistically significant only in the cases when ossification centre was ossified while epiphyseal cartilage was not which corresponded to second stage. Unfortunately, although the survey encompassed both body sides the authors did not provide statistical analyses of side variation among their sample.

The study conducted on Australian population was published in 2011 by Bassed et al. (40) (Table 19). The authors used methodology previously developed by Schmeling et al. (25). The not ossified epiphyseal cartilage appeared across the entire male sample, while in females it could be observed until the age of 21. The signs of partly ossified epiphyseal cartilage were detected in the age range between 17 and 25 years in both sexes. Finally, the earliest age of fully ossified epiphyseal cartilage was 17 years in males and 19 years in females. Although, the authors obviously analysed body sides they however did not provide any statistical information about interside variation. In addition, the earlier maturity of female population was observed.

Although Schulz was the first who recommended slice thickness, his and Kreitner's publications were criticized because of wide slice thickness ranges of encompassed samples (8, 39-41). Mühler et al. even went step further, evaluating age estimation process on different slice thicknesses among same individuals (41). The authors observed higher ossification stages at increased slice thicknesses. This was explained by arousal of partial volume effect which was able to partly or fully mask fine radiological features that are mandatory for stage distinction. Therefore, increasing of slice thickness led to identification of different ossification stages in the same cases. Overall, in order to achieve maximum accuracy the authors recommended 1mm thickness for CT examinations.

The Question of Epiphyseal Ossification Timing Comparability

Unfortunately, in some countries including Serbia, CTs are available only at clinics and not at institutes for research or forensic purposes. Therefore, the testing of developed age estimation methods and its adjustments to a specific population requires usage of CT examinations performed on living individuals. The standard clinical protocols are always in concordance with ALARA ("as low as is reasonably achievable") principle (42). Therefore, as slice thickness is defined as a dose-related parameter, in the current study the available CT protocol involved nominal slice thickness of 5mm with reconstruction to 3mm (43-45).

Although present study tested five-stage scoring system on CT studies with thicker slices than recommended, the results were fairly in concordance with the literature (Table 19). Thus, the first stage was recorded until the age of 17 which was similar to Kreitner and Kellinghaus who both reported 16 years of age (18, 39). Nevertheless, Bassed et al. published somewhat higher age limits in males and females 21 and 19 years of age, respectively (40). Unfortunately, other authors did not provide information on their results concerning the first stage (31, 38). The signs of the second stage could be seen until the age of 18 in the current study. Similarly, Kellinghaus reported the upper limits at 20 and 19 years of age in males and females, respectively. However, the results of other studies (18, 38, 40) were in the boundaries between 21 and 25 years of age with exception of Schulz who did not provide necessary data (31). According to the results of the present study, the signs of partly ossified epiphyseal cartilage were observed in the range between 15 and 25 years, which was almost absolutely in concordance to the all cited authors (18, 31, 38-40). In addition, Schulz published only the earliest age of partly ossified epiphyseal cartilage without providing any data about the latest age (31). The earliest age of completely ossified epiphyseal cartilage of 17 years was recorded in Australian male population (40), while all other studies including the current study reported higher boundary. Namely, the present study and Schulze's survey (38) recorded the first signs of completely ossified epiphyseal cartilage at the age of 19. The same age was observed in the female Australian population (40), while the published results of other studies set the age boundary at 21-22 years of age (18, 31, 39).

Table 19. Comparative overview of the epiphyseal union stage timing through different studies with applied CT methodology.

Study group	Year	Population	Staging System	Sample Size (N)	Males (N)	Females (N)	Age Range (in years)		Latest Age of Not Ossified Epiphyseal Cartilage Male/Female (in years)	Earliest Age of Partly Ossified Epiphyseal Cartilage Male/Female (in years)	Latest Age of Partly Ossified Epiphyseal Cartilage Male/Female (in years)	Earliest Age of Completely Ossified Epiphyseal Cartilage Male/Female (in years)
							min	max				
The Current Study	2013	Serbian	5-Phase	154	97	57	15	35	18	15	25	19
Bassed R. B. et al. (40)	2011	Australian	5-Phase	674	455	219	15	25	25/21	17	25	17/19
Kellinghaus M. et al. (39)	2010	NA	5-Phase	592	288	214	10	35	20/19	18/17	26/26	22/21
Schulze D. et al. (38)	2006	NA	4-Phase	100	50	50	16	25.9	24	16	25	19
Schulz R. et al. (31)	2005	European	5-Phase	556	417	139	15	30	NA	17/16	NA	21
Kreitner K.F. et al. (18)	1998	European	4-Phase	380	229	151	0	30	22	16	26	22

NA – Not Available.

The observed slight discrepancy in ossification timing could possibly be aroused not only by different slice thicknesses, but also by different sample population. Conversely to macroscopic analyses, the author of the current study could not compare actual results to some socioeconomically similar population. Even more, Kellinghaus and Schulze did not provide any information about nationality or socioeconomic status of encompassed population (38, 39). One could speculate that their samples were derived from German citizens and therefore belong to European population like in the studies of Kreitner and Schulz (18, 31). However, according to the official Eurostat data (46) Germany is the country with the highest rate of foreigners who are mostly citizens of non-EU countries. Furthermore, a half of foreigners in European Union derive from the countries with medium human development index (HDI), about a third from high HDI countries, while only 3.1% originates from the candidate countries which correspond to our population (46). Overall, although Eurostat classified Serbian population separately to candidate countries, the data might be comparable as UNDP ranked Serbia in countries with high HDI (27).

The Role of Clavicular CT Morphology

The analyses of clavicular shaft revealed broad range of variety data which resulted in inability to create appropriate age-related patterns. The analyses only detected correlation between the acquired measurements and individual's age among males. Thus, calculated anterior to posterior cortical thickness ratio, diameter of medullar canal and clavicular shaft were age-related. However, the further application of the tested radiological features could not be advisable in the process of age estimation.

The Usefulness of Medial Clavicular Density

Heretofore, the only developed histological methods for clavicles were based on the osteon population densities in the clavicular mid-shaft in the process of age estimation (19, 23). Conversely, the current study investigated histomorphometric parameters of medial clavicular end similarly to the almost every single previously developed macroscopic age estimation method related to medial clavicles. The involved histomorphometric analyses were similar to others which were previously applied in various skeletal sites in order to assess age-related deterioration of bone micro-architecture, particularly in femur and vertebrae (21, 22). In the present study trabecular bone volume fraction and trabecular width linearly declined with age in males, demonstrating significant age-related deterioration of trabecular bone in the medial end of clavicle in men. The results also suggested that quantitative architectural parameters of trabecular bone may represent an age-distinctive feature in clavicles.

However, the histomorphometric analyses are available only in cadaver studies, while justification for their use in a living is highly questionable. Nevertheless, it is known that the quantity of trabecular and cortical bone influence bone density which relatively could be measured by CT (47-50). This method is practical and easily achievable, and therefore it could be used as an additional tool in age estimation process. The results of the present study provided several equations based on estimated correlation between individual's age and bilaterally observed radiodensity of the sternal epiphyseal-metaphyseal region in the whole sample, as well as in the male part of the sample where the analyses were conducted without side differentiation.

The Significance of the First Costal Cartilage Involvement

The analyses of radiodensity were also conducted on the first costal cartilage. As it was expected the signs of mineralization and ossification altered its radiodensity which expressed statistically significant correlation to individual's age. The first costal cartilage already received considerable attention as an age indicator in the large body of literature. However, the only previous study with applied CT methodology was published in 2010 by Moskovitch (24). The survey encompassed CT studies of 160 individuals, whose age ranged from 15 to 30 years. The authors conducted three- and two-dimensional analysis. Interestingly, the published results of the two-dimensional analyses revealed new age related features which cannot be seen by direct skeletal inspection. Actually, the authors observed osseus and calcified projections (OCP) within costal cartilage. The survey also presented a new staging system which was based on several features of OCPs. Actually, the presence, length and shape of OCPs were scored. Thus, zero was assigned if OCP were not observed. Otherwise, if linear projections were recorded and its length did not exceed 50% of distance between the costal face and sternum, score was set at one. However, if its length exceeded 50% of previously defined distance, score was set at two. The nonlinear appearance of calcifications projecting medially toward sternum was ascribed to the maximal score of three. According to the results, several conclusions were developed: OCP's were absent until the age of 25 in males; females with observed two OCPs could only be older than 20 years of age; a nonlinear appearance of extensive cartilaginous calcifications was observed after the age of 20 and 25 in males and females, respectively. However, although results were present separately for males and females, the conducted statistics did not reveal sexual dimorphism. Unfortunately, analyses of body side variation were not provided as study sample involved only right side ribs.

The proposed first cartilage-staging system (24) was for the first time tested in the present study. Although both studies agreed that OCP's features correlate with age, the results were somewhat different. Thus, in the present study, single OCP and two OCPs, were observed at the same age and this detection was made in 19-year-old-individual, while the non-linear appearances of calcifications were noted after the age of 23. Conversly to Moskovitch, who reported absence of OCPs until the age of 24 and 26 in males and females, respectively, the absence of linear projections in the present study was noted in almost all encompassed ages, from 15 to 34 years of age. Still almost half of all those observations were made in the age range between 15 and 18 years. Overall, the present study estimated statistically significant correlation between individual's age and calculated stages of the OCPs. However, the results did not reveal satisfying distinction between all stages, except for the first stage. Therefore, the author additionally presented the equation based on correlation of individual's age to calculated relation of projection's length to distance between costal face and sternum.

The results of the current study suggested that the mutual enrollment of the applied clavicular and the first costal cartilage scoring systems would not benefit in age estimation process. However, the interaction between radiodensity of the sternal epiphyseal-metaphyseal region and the first costal cartilage represented an interesting new age predictor, which was more reliable in comparison to all other tested measurement-based predictors.

The Question of Intersex Variability

Even though the literature stayed ambiguous whether sexual maturation has any influence on the epiphyseal union progress, the current study identified females' tendency towards the higher epiphyseal union stages which was statistically significant only in macroscopic part of the study. Similar findings were previously reported by Singh and Chavali (11). Furthermore, the earlier maturity of female population was also observed by CT, according to Bassed et al. (40). The differences among the sexes in some phases of epiphyseal union were reported by Schmeling et al. (25) who developed five-stage scoring system based on applied conventional radiology method. Afterwards, statistically significant intersex variability was detected only in the second stage of epiphyseal cartilage ossification process according to the surveys of Schulz et al. (31) and Kellinghaus et al. (39) which were based on CT methodology with applied Schmeling's five-stage scoring system. However, the results of the present study as well as Kreitner et al. (18), and Schulze et al. (38), could not detect inter-sex variation. Nevertheless, the current study identified several macroscopic morphological features with expressed sex-dependence. For instance, the morphology of the notch for the first rib, the margin of the articular surface, as well as basic morphology of articular surface differed significantly between male and female samples. Moreover, sex difference was ascertainable in two microscopic characteristics: trabecular bone volume fraction and minimum trabecular width. The sex dependent features were also detected by CT. Thus, intersex variability was observed in following variables: thickness of anterior cortex of the right side clavicles, calculated anterior to posterior cortical thickness ratio, diameter of the medullar canal, diameter of the clavicular shaft, and calculated medullar to shaft diameter ratio. Conversely, the conducted analyses confirmed sexual equality among the observed features of the first cartilage which was previously reported by Moskovitch (24).

The Presence of Bilateralism

Heretofore, all conducted studies which involved analyses of bilateralism did not report statistically significant signs of side variability (7, 11, 18, 31, 38). Those results corresponded to the analyses of the present study which also could not detect side disparity concerning the ossification stages of the medial clavicular epiphyseal cartilage. Nevertheless, the newly introduced features of CT analyses which correlated with age expressed body side discrepancy. Thus, according to the present study, such features involved radiodensity of the sternal epiphyseal-metaphyseal region of the clavicle, and acquired diameter of the medullar canal. In addition, the applied tests did not reveal signs of bilateralism in tested sample of the first costal cartilage.

The Reproducibility

Overall, the estimated interobserver reliability of observed macroscopic and radiological stages as well as acquired quantitative measurements was in the range of fair to almost perfect agreement. Therefore, the current study demonstrated satisfying reproducibility of tested age estimation methodology.

Conclusion

The macroscopic part of the current study, apart from the epiphyseal union timing, managed to identify several other age-related features which could be applied as additional guidance for age estimation and that could be easily identified by naked eye. Among investigated morphological features, signs of lipping in the region of the notch for the first rib as well as exostoses and bone overgrowths of the articular surface margin should be considered as age-depended attributes of medial clavicles and indicative for the age beyond 44 and 53 years, respectively.

The results also suggested trabecular bone volume fraction and minimum trabecular width as age-distinctive microscopic features of medial clavicles in men. Those findings and the further CT analyses of the sternal epiphyseal-metaphyseal region led to the implementation of several age related equations. Beside radiodensity and the stages of epiphyseal cartilage ossification of medial clavicles, the study also detected other age-related features among males, such as calculated anterior to posterior cortical thickness ratio or for instance, diameters of the medullar canal and clavicular shaft.

Although the analyses of the first costal cartilage estimated statistically significant correlation between individual's age and calculated stages of the OCPs, the distinction between the stages was not satisfying.

The interaction between applied scoring systems for the ossification status of the medial clavicular epiphyseal cartilage and OCP's stages was not statistically significantly influenced by age, and their mutual enrollment might not be advisable. However, the results of the current study suggested that acquired radiodensity of the sternal epiphyseal-metaphyseal region and radiodensity of the first costal cartilage stood out as an interesting new age predictors with mutual relationship which could represent an additional usuful tool in future analyses. The conducted statistics allowed creation of the reliable equation:

$$Age = 0.035 \cdot FC.Dn - 0.039 \cdot Ep.Dn + 30.831$$

Which was even more pronounced after the process of data normalization:

$$Age = 5.695 \cdot \ln FC.Dn - 0.037 \cdot Ep.Dn + 7.534$$

In addition, evaluation of sex differences in the observed macroscopic features revealed that epiphyseal union timing, morphology of the notch for the first rib, margin of the articular surface, and basic morphology of articular surface were sex-dependent attributes. Moreover, sex difference was ascertainable in two microscopic characteristics: trabecular bone volume fraction and minimum trabecular width. Intersex variability was also observed by CT in several age-related features: calculated anterior to posterior cortical thickness ratio, diameter of the medullar canal, and diameter of the clavicular shaft. Finally, the current study identified females' tendency towards the higher epiphyseal union stages.

Overall, this is the first study that has been carried out in a Balkan population with the aim to examine whether morphological, radiological and histological analysis of the medial clavicles as well as radiological examination of the first costal cartilage could be applied with success in age assessment of individuals in anthropological and forensic practice. Although, considerable overlapping and inconsistent age- and sex-dependence of analysed morphological, histological, and radiological features precluded independent creation of more accurate age-specific phases and sex-dependence criteria, multifactorial approach could be suggested as more reliable one.

The provided results could especially benefit the age estimation process applied in cases of advanced decomposition state narrowing and leading investigation. However, the future researchers should consider that radiological analyses encompassed only living individuals and therefore the obtained results should be used with caution in cases of advanced decomposition as the influence of the natural process of decay is yet to be tested. Therefore, the provided conclusions should be applied as an additional guidance for age estimation in each specific case.

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Short Professional Biography

Petar Milenković was born in Belgrade, in 1982. After finishing elementary and high school in Belgrade, he was enrolled in School of Medicine, University of Belgrade, in 2001. Since February 2003 he was involved in the research team of Laboratory for Anthropology Institute of Anatomy lead by Professor Marija Djuric. Entailed actively in analyses of skeletal remains, observing and processing results, as well as critical analyses of literature during preparation of scientific papers.

He graduated from School of Medicine in 2008 with GPA 9.40. Afterward, in 2009, he was enrolled in Doctoral Studies at School of Medicine, University of Belgrade – course: Skeletal Biology. During the undergraduate and postgraduate studies he received Scholarship of the Republic Foundation for Development of Scientific and Artistic Youth. He became radiology resident of Institute for Oncology and Radiology of Serbia at School of Medicine, University of Belgrade, in November 2012.

Прилог 1.

Изјава о ауторству

Потписани Petar P. Milenković

број уписа ______________________

Изјављујем

да је докторска дисертација под насловом

Age Estimation Based on Analyses of Sternal End of Clavicle and the First Costal Cartilage
(*Procena godina starosti osobe analizom unutrašnjeg okrajka ključne kosti i hrskavice prvog rebra*)

- резултат сопственог истраживачког рада,
- да предложена дисертација у целини ни у деловима није била предложена за добијање било које дипломе према студијским програмима других високошколских установа,
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио интелектуалну својину других лица.

Потпис докторанда

У Београду, 18.02.2014.

Прилог 2.

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора Petar P. Milenković

Број уписа

Студијски програм Skeletal Biology (Biologija skeleta)

Наслов рада Age Estimation Based on Analyses of Sternal End of Clavicle and the First Costal Cartilage (*Procena godina starosti osobe analizom unutrašnjeg okrajka ključne kosti i hrskavice prvog rebra*)

Ментор prof. dr Slobodan Nikolić

Потписани Petar P. Milenković

изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла за објављивање на порталу **Дигиталног репозиторијума Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског звања доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

Потпис докторанда

У Београду, 18.02.2014.

Прилог 3.

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

Age Estimation Based on Analyses of Sternal End of Clavicle and the First Costal Cartilage

(*Procena godina starosti osobe analizom unutrašnjeg okrajka ključne kosti i hrskavice prvog rebra*)

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигитални репозиторијум Универзитета у Београду могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

1. Ауторство
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5. Ауторство – без прераде
6. Ауторство – делити под истим условима

(Молимо да заокружите само једну од шест понуђених лиценци, кратак опис лиценци дат је на полеђини листа).

Потпис докторанда

У Београду, 18.02.2014.