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TITOLO TESI

Age estimation and sex determination in human skeletal remains. A test of the common methods used in anthropology for sex determination and age estimation applied to identified human European skeletal collection (Bologna, Coimbra, 19th -20th c.)

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SUMMARY

CHAPTER 1: Introduction

Age estimation and sex determination are the first analysis that are performed when human skeletal remains are analysed.

Age and sex assessment are essential not only for the study of biological characteristics that can display different distribution in relation to sex and age (e.g. paleonutritional and paleodemography markers), but also to define the biodemographycal structure of the population. However, sometimes this analysis can be more difficult or limited because of the incompleteness of the studied specimens. This is very common in the study of archaeological samples.

When studying ageing human remains, it has to be kept in mind that the age estimation from the skeleton represents the biological age and not the chronological one, in fact biological age and chronological age are usually correlated but do not always coincide. When discussing biological age it must be noted that it refers to the discrepancy between an individual's bone developmental stage and his/her chronological age, the latter being unknown for most skeletal populations. This differentiation is the subject of the research made by human biologists (Bittles and Collins 1986) and paleodemographers (Paine, 1997).

1.1) Age determination

Methods for age estimation are based on stages of skeletal and dental processes of development for sub-adult individuals and senescence and degeneration for adult individuals.

The methodologies used to determine age in growing individuals are considered to be quite reliable because of their focus on the observation of skeletal changes following a predictable pattern, that occur in the same order in all individuals (Stevenson, 1924; Flecker, 1942; McKern and Stewart, 1957; Ubelaker, 1978; Fazekas and Kosa, 1978; Hoffman, 1979; ecc.).

A lot of macroscopic and microscopic studies were developed to determine the age from the adult skeleton: cranial suture closure (Meindl and Lovejoy, 1985; Mann et al, 1987; Mann et al, 1991), morphological changes of the sternal end of the fourth rib (Iscan et al., 1984, 1985, 1986), of the pubic symphysis (Snow, 1983; Suchey and Brooks, 1990; Murray et.al 1991; Klepinger et al. 1992) and of the auricular surface (Lovejoy, et al. 1985); microscopic analysis of bone (osteon counting) (Stout, 1986, Nelson, 1992, Dudar et al. 1993); dental wear (Brothwell, 1981; Lovejoy, 1985).

The methods that have been developed to determine age in adult individuals are focused on the analysis of macroscopic and microscopic changes, on the process of senescence and its consequent degenerative changes in the skeleton and dentition. The aforementioned changes are influenced by a complex interaction between exogenous and endogenous factors. The complicated interaction among genes, culture, and the environment, determines the rate and degree of changes in individual life history (Harper & Crews, 2000; Schmitt et al., 2002) and substantially affects the assessment of age at death. These factors generate a wide variability within and among populations (Todd, 1920; Kerley, 1965; Ahlqvist and Damsten, 1969; Meindl and Lovejoy, 1985; Meindl et al, 1985). Furthermore, developmental and degenerative processes affect different anatomical structures in different ways in the individual life history.

Most of the methods and their modifications have been developed and tested only on a few population samples from the late 19th century to present age and mainly on North American and European populations. However, some studies have tested different age estimation methods on population from other geographic areas (Santos, 1992; Saunders et al. 1992; Schmitt, 2004; Sakaue, 2006; Hens, 2008; Rivera - Sandoval, 2014). The reliability of methods for estimating age at death in skeletal samples has been demonstrated to be higher in skeletal series from the populations on which the methods were developed (Schmitt et al. 2002; Baccino et al. 2006).
Researchers have raised various questions: the applicability of various methods on different populations (Iscan et al., 1987; Oettle and Steyn 2000; Schmitt 2004) and on individuals of various age ranges (Lovejoy et al., 1985; Saunders et al., 1992), the effect of inter- and intra-observer error (Bedford et al. 1993), and the use of different statistical strategies (Lovejoy et al., 1985; Saunders et al., 1992) leading to difficulties in comparing results when different samples, methods, and statistical procedures, are used.

The methods based on changes on the pubic symphysis and the auricular surface are the most commonly used in bioarchaeology to determine age at death (Todd, 1920; Hanihara, 1952; McKern and Stewart, 1957; Nemeskeri et al., 1960; Gilbert and McKern, 1973; Suchey, 1979; Meindl et al., 1985; Katz and Suchey, 1986; Zhang, 1986; Brooks and Suchey, 1990; Klepinger et al., 1992; Sinha and Gupta, 1995; Hoppa, 2000) and have been developed and assessed on modern skeletal samples (from later 19th century to present age) derived from North American populations (Gilbert & McKern, 1973; Murray et al. 1991; Saunder et al. 1992; Klepinger et al., 1992; Bedford et al., 1993).
The anatomist Todd (1920) published the first systematic study about age changes in the pubic bone, basing his study on a large sample (n=306), from a skeletal series collected between 1912 and 1920 in Cleveland, Ohio (now called the Hamman-Todd Collection).
However, there are problems with the ages, some skeletons were deleted if not fitting standards for skeletal development, reducing the sample variability. Even the distribution of age is uneven: there are many peaks at 35, 40, 45, 50 and 60 years in the white male sample; the black male sample shows this feature but to a lesser degree. Todd points out that this peaks may be a result of some rounding off of the ages. (Todd, 1920: 289,314)
Brooks (1984) notes on the male sample that “Many of them were transients, with no known age data. Their age estimation was made by Todd or one of the anatomists when the cadaver was being prepared for the WRU Medical School dissection classes”.
McKern & Stewart (1957) studied a sample of 349 American soldiers killed in the Korean War. The authors divided the symphysial face into three-component: the dorsal plateau, the ventral rampart and the symphysial rim and developed a six stage system for each component to age estimation. The ages in that sample should be really accurate, because it come from United States government records.

Previous works have argued the possible influences of population variation on age estimation based on the **pubic symphysis** (Todd, 1920, 1921; McKern and Stewart, 1957; Meindl et al., 1985;Katz and Suchey, 1989; Sinha and Gupta, 1995; Hoppa, 2000; Schmitt, 2004), the most widely used method is Suchey Brooks (Brooks and Suchey, 1990); many later studies tested and modified this method (Klepinger et al. 1992). Katz and Suchey (1989) observed an increase of the differences during individual life among populations reporting significant differences after phase III (21-53 years for the female and 21-46 for the male). At the same time, Klepinger et al. (1992) suggested a "racially specific" refinement of the Suchey–Brooks system. However, Katz and Suchey (1989) and Suchey and Katz (1998) cautioned about the impossibility of a precise evaluation of the causes of differences observed. In fact, they might be related to genetic or environmental factors such as nutrition or drug/alcohol use. However, for American samples, this method is considered the best one for the age at death estimation of skeletal remains and it is routinely used in forensic cases and bioarchaeological contexts (Klepinger et al. 1992; Jackes, 2000; Ubelaker, 2000; Byrs, 2002). In spite of its popularity, age determination from the pubic symphysis has not received complete support in literature based on modern French autopsy individuals (Baccino et al. 1999) and Canadian pioneers (Saunders et al., 1992), reporting problems in determining age in individuals over 35 years old.

Another method widely used to age human remains is based on the study of the changes of the **auricular surface** of the ilium. Since 1930 the morphology of the auricular surface of the ilium has been considered related to the individual senescence. However, it is only in 1983 that Meindl and his collaborators started studying this area as an age at death indicator. In 1985, Lovejoy and co-workers developed a new method using archaeological samples, (250 individuals from Libben collection, and approximately 500 American from the Hamann-Todd collection). In archaeological contexts, the auricular surface is often better preserved than the pubic symphysis and the morphological changes are protracted to the sixth decade of life.

On the other hand the application of the Lovejoy method presents a higher degree of difficulty than the Suchey-Brooks method for the pubic symphysis. Moreover some studies have highlighted repeatability problems which affect the auricular surface method (e.g., Murray et al. 1991; Falys et al. 2006).

The assessment of the age through the **cranial suture closure** analysis, although widely used, is controversial; Krogman concluded in 1962 that suture closure seemed promising to use to estimate age even though this method lacks of an appropriate number of studies of suture obliteration (Krogman 1962).

By the end of the 19th century, studies of cranial suture closure had been conducted by many researchers (Broca, 1861; Ribbe, 1885; Schmidt, 1888; Dwight, 1890a; Parsons and Box, 1905). These early researchers found a positive correlation with age commencing with basilar suture (synchondrosis) closure at 18-21 years followed by observations of the vault beginning endocranially anywhere between 25 and 40 years of age and continuing through the sixties. However, the extreme variability in the order and timing of closure was noted (Dwight, 1890a).

Todd and Lyon (1924) were among the first to use a scoring system: each suture sites were recorded and a score from zero (no closure) to four (complete closure) was given to each suture. The different landmarks on each suture (i. e. pars bregmatica, pars vertices, pars obelica, and pars lambdica) were averaged together and observed individually for patterns of closure. Using this system, they observed that endocranial suture is more reliable from 26-30 years of age. Additionally, the ectocranial sutures are highly unreliable for age estimation. After the works of Todd and Lyon (1924), criticism of suture closure as an indicator of age continued and cranial suture closure became more of a general age indicator and the last resort for individual identification. Acsadi and Nemeskeri (1970) observed that there is a uniform trend of rapid closure early on followed by a slower and gradual closure with advancing age. Perizonius (1984) observed that all sutures especially the coronal, exhibited a significant degree of positive correlation with age, in the 20-49 years of age. He also added that there is a negative correlation with age from 70 to 79 years.

Mendl and Lovejoy (1985) have tried to improve the scoring system, and have concluded that cranial suture, combined with other age indicators, may be more reliable for forensic purposes.

The development of unbiased and definite criteria for using sutures in age determination has been difficult (Acsadi, Nemeskery 1970; Perizonius 1984; Meindl et al. 1985; Hauser 1991; Key et al. 1994). Some studies have shown that its results are largely unreliable and irreproducible, and the correlation between suture closure and age is medium to poor (Singer 1953; Powers 1962; Gruspier and Mullen 1991; Hershkovitz et al. 1997; Galera, Ubelaker, and Hayek 1998; Ginter 2005; Beauthier et al. 2010). Other sutural systems, such as the frontosphenoidal sutures (Dorandeu et al. 2008, 2009) frontonasal sutures, (Alesbury et al. 2013) and sutures of the maxilla and palate (Sejrsen et al. 1993; Wehrbein et al 2001) have been investigated by several researchers for purposes of age estimation but they are not widely used.

In conclusion Garvin et al. (2012) indicate that the Suchey-Brooks pubic symphysis method (1990) remains the most highly preferred ageing technique. For the cranial sutures the most common use method was Meindl and Lovejoy (1970). However several researchers agreed with the use of cranial suture only when other age indicators are not available (Garvin et al. 2012).

The methods concerning age estimation by tooth wear were not included because the correlation between wear, nutrition and non-masticatory activities; such methods are sometimes used in bioarchaeology but mainly on homogeneous samples from the same archaeological contest as support to other methods and to estimate its age.

1.2) Sex attribution

The functional modification and the evolutionary adaptation are indicated by sexual dimorphism in the pelvis. The male pelvis evolution ensured the bipedality success, while the female pelvic structure modification ensured the obstetric success despite the increase in size of the head of the newborn through human evolution (Cox, 1989; Tague, 1995). Male individuals presents a high and narrow pelvis structure whereas female individuals present a transversal oval pelvic diameter with a relatively wider inlet. These aspects provide an osteological sex indicator.

In the human skeleton and dentition sexual dimorphism can be well established and can be studied in detail (e.g. Keen, 1950; Giles and Elliot, 1963; Frayer and Wolpoff, 1984; Konigsberg and Hens, 1998; Graw et al.,1999; Rosas and Bastir, 2002; Correia et al., 2005; Schwartz and Dean, 2005). An accurate determination of the sex when analysing human remains is essential when the biology and behaviour of past populations has to be reconstructed (Frayer and Wolpoff, 1984; Walrath et al., 2004).

Sex is generally inferred by using skeletal morphology, but in recent years DNA has also been used in forensic studies, and, sometimes, in the bioarchaeological field (Stone et al. 1996; Stone, 2000, 2008).

However, when analysing human remains, sex determination can be complicated by a considerable overlap in the size and degree of robustness both in male and female skeletons. Furthermore, a higher level of robustness, which affected the mandible and the skull in past populations, led to a misunderstanding of sexual dimorphism in the past (Weiss, 1972). Even in more recent populations the environmental and behavioural influences can have effect on the final robustness of the skeleton, resulting possibly in an incorrect determination of sex (Krogman and Iscan, 1986; Novotny, 1986; Hoyme and Iscan, 1989; Novotny et al., 1993; St. Bruzek, 1992, 1995, 2002).

Moreover the androgen levels in the male individuals vary with age, in particular before puberty. This means that pre-pubertal sexual dimorphism in the skeleton is light, therefore the determination of sex in the juvenile individuals is much more difficult than in adults.

When analysing human remains, different bones can be potentially used but the most significant for sex estimation purposes are pelvis and skull. Accuracy of sex determination is better from the pelvis (Ferenbach et al. 1980; Krogmanand and Iscan, 1986; MacLaughlin and Bruce, 1990; Walrath et al., 2004) however the skull can be used as well in archaeological contexts as it is usually better preserved (Novotny et al., 1993).

Nevertheless, sex determination based on the skull presents a high grade of misattribution (20% of specimens) (Masset, 1987).

Meindl et al. (1985) suggested that this error in sex determination origins from inherent variability of the dimorphism of the skull, compared to the pelvic dimorphism due to the selective effects of childbirth.

Size is one of the most important factors influencing sex differences in the cranium (Uytterschaut, 1986; Wood et al., 1991; Rosas and Bastir, 2002). Sex differences influence also the development of bony structures such as the superciliary arches, glabellar region, mastoid process and aspects of robusticity (Ascàdi and Nemeskèri, 1970).

Moreover according to the great intra population variability which usually is higher than the inter population variability (White, 1991; Barbujani, 2006) some characteristics present an ambiguous morphology or even not sex-according morphology.

1.3) Indentified collections

The identified collections are essential to the development of methods for estimating the age and sex determination. The methods for the age estimation and sex determination are elaborated on these collections based on the observation of morphological changes of some specific skeletal and dental markers following the ageing process.

There are different kinds of identified collections:

a) the collections housed in the Departments of Anatomy consisting of individuals from room of dissection. These bones can belong to poor people, non-claimed corpses or people who gave their body to the research i. e., the osteological collectios of Hamman-Todd (Museum of Natural History of Cleveland, Ohio) and the Terry Anatomical Collection (National Musum of Natural History, Smithsonian Institution, Washington);
b) collections consisting of bones of people with some skeletal pathologies, used for didactic and research purposes, i.e. the collection of "National Museum of Health and Medicine" (Washington) and the Museum of Pathologic Anatomy of Wien;
c) collections consisting of people involved in forensic cases like "William M Bas Donald Collection and Forensic Anthropology Data Bank" (University of Tennesse) and "FACES Laboratory Collection" (Louisiana State University);
d) collections consisting of identified individuals from cemeteries, i.e., the osteological collection of Sassari (or Frassetto collection) and Bologna (Museo di Antropologia – BiGeA- University of Bologna, Italy) and the collection of Coimbra (Museum of Anthropology University of Coimbra, Portugal).

Whatever the origin of the human remains, it is important that the samples used as reference to elaborate and to test methods for the age estimation and sex determination satisfy important requirements connected to different elements: sample dimension, numerical representativeness for the age group and sex, geographical origin and socio-economic conditions of people.

We consider as "identified" an osteological collection where one or more elements allowing the individual identification are associated, e.g. the labels on the coffins reporting name, sex, and age at death, baptism, matrimony and death certifications, medical records, etc.

In some of these collections used as reference the age information may be ambiguous (e.g. collection of Hamman-Todd; Terry collection). For example, in the Hamman-Todd collection there are only 512 individuals properly identified in relation to age-at-death, and a group of esteemed round off age individuals (-5; -0) - as they or their relatives reported at the moment of the hospital admission (Todd, 1920) - in a total of 3422 individuals. Moreover, the anatomists changed in several cases the age reported on documents after the morphological examination of the tissues and of skeletons (Lovejoy, 1985a).

Besides the problems about information, there are issued related to the dimension of the sample and its comparability with age, sex, geographical origin and socio-economic conditions of the individuals (Bocquet, 1977).

Bocquet – Appel e Masset (1982) underlined for the first time the importance of the sample reference to elaborate the "standard" of the method for the age estimation. The structure of the reference sample might be the reason of the "attraction of the middle", which is a phenomen observed in a lot of cases irrespectively of the selected marker, that consists in the overestimation of the youngers and in the underestimation of the older (Masset, 1989; Murray e Murray 1991; Bedford et al., 1993).

Moreover the identified collections of individuals must be representative of the population features: socio-economic status, health conditions, geographical origin. However, the osteological samples in University or medical institutes are assembled following criteria that favour some form of selection: the Hamman-Todd collection consists of poor and not reclaimed individuals from dissection rooms; the Terry collection consists of not reclaimed and poor individuals, with a prevalence of African American and old individuals, therefore it might not be representative neither of the whole American population nor of the population of Sant Louis, even though it is one of the largest (1600 skeletons) and well documented (Galera et al. 1998).

The 202 individuals of the Grant Collection belong to individuals from local hospitals and social institutes who gave their body to the department of Anatomy of the University of Toronto. Most of them were white European people and the collection mostly consists of male individual (N=175) forty years and older (N=147) seasonal workers, recent immigrants without family.

Collections or subsamples can be also actively selected eliminating those individuals who have morphological features different from their contemporaries in order to elaborate standards. This technique leads to reduce the variability of the sample (Todd, 1920).

A short description of some of the most studied human skeletal collection can be found in tab. 1

IDENTIFIED HUMAN SKELETAL COLLECTIONS							
COLLECTION	**Bologna Collection**	Sassari Collection	**Coimbra Identified Skeletons Collection**	Luís Lopes Collection (Lisbon Collection)	Spitalfields Coffin Plate Collection	Wendorf Skeletal Collection	St. Bride's Church Documented Collection
INSTITUTION	Museum of Anthropology, University of Bologna, Italy	Part of the Frassetto collections, Museum of Anthropology, University of Bologna, Italy	Department of Anthropology, University of Coimbra	Department of Zoology and Anthropology, National Museum of Natural History (Bocage Museum)	Natural History Museum of London	epartment of Ancient Egypt and Sudan, British Museum	St. Bride's Church, Fleet Street, London
LOCATION	Bologna, Italy	Sardinia, Italy	Coimbra, Portugal	Lisbon, Portugal	London, England	London, England	London, England
SAMPLE	The collection is compose by 296 individuals died in a period ranging from 1898 to 1944.	Contains complete skeletons from 606 individuals who died in the early 20th century and were buried in local cemeteries	Composed of 505 identified skeletons. Dates of birth range from 1826 to 1922, and dates of death range from 1904 to 1938.	Consists of 1,692 identified skeletons from modern cemeteries in Lisbon, Portugal. Dates of birth range from 1805 to 1972 and dates of death range from 1880 to 1975.	Contains 968 individuals excavated from the crypt at Christ Church, Spitalfields, dating from 1729 to 1859 AD.	This collection houses skeletal remains excavated from five archaeological sites in Egypt and Sudan.	Contains 244 identified sets of remains Ages at death range from 3 days to 91 years.
INFORMATION	Sex, age,occupatio, cause of deathn and date of birth and death are available for individuals.	Sex, age, and date of death are available for most individuals, and date of birth and occupation are available for many.	Occupations and causes of death are available.	Demographic data available includes age at death, date and cause of death, place of birth, occupation, and place of residence.	Information about the individuals in the collection stems from coffin plates associated with them.	Information available for each individual includes context, sex, age, stature, preservation, and pathological conditions.	Collection of 18th and 19th century skeletons from the crypt at St. Bride's Church, Fleet Street, London. Documentation comes from original coffin plates which listed ages and sexes.

Table 1: a short description of some of the most studied human skeletal collection.

IDENTIFIED HUMAN SKELETAL COLLECTIONS							
COLLECTION	Wendorf Skeletal Collection	St. Thomas Anglican Church	University of Athens Human Skeletal Reference Collection	Pretoria Bone Collection	Raymond A. Dart Collectio of Human Skeleton	Robert J. Terry Anatomical Skeletal Collection	William M. Bass Donated Skeletal Collection
INSTITUTION	Department of Ancient Egypt and Sudan, British Museum		Department of Animal and Human Physiology, University of Athens	Department of Anatomy, School of Medicine, University of Pretoria	Housed in the School of Anatomical Sciences at the University of the Witwatersrand	Department of Anthropology, National Museum of Natural History	Department of Anthropology, University of Tennessee, Knoxville
LOCATION	London, England	Belleville, Ontario	Athens, Greece	Pretoria, South Africa	Joannecburg, South Africa	Washington, DC, USA	Knoxville, Tennessee, USA
SAMPLE	This collection houses skeletal remains excavated from five archaeological sites in Egypt and Sudan.	The excavation of delimitated area yielded 595 individuals.	Contains 225 modern skeletons from the mid to late 20th century from cemeteries around Athens, Greece.	It contains 290 complete skeletons, 704 complete skulls, 989 incomplete skulls, 541 complete postcranials, and 418 incomplete postcranials.	A recent inventory the Dart Collection currently comprises 2,605 skeletons.	This collection consists of 1728 individuals	Contains around 900 adult individuals along with some infant and fetal remains and cremains.
INFORMATION	Information available for each individual includes context, sex, age, stature, preservation, and pathological conditions.	Parish records	Documentation is available for almost all individuals and includes age, sex, occupation, and cause of death.	This collection is composed of the skeletal remains of individuals who died post 1942 whose bodies were either unclaimed or donated to the University of Pretoria, and demographic information available includes age, sex, and population affinity.	The recorded ages at death range from the first year to over 100 years of age, but the majority of individuals died between the ages of 20 and 70.	Dates of birth ranging from 1822 to 1943 and ages at death ranging from 16 to 102 years. Demographic information includes age, sex, ancestry, cause of death, and pathological conditions.	Demographic data is available for most individuals and includes age, sex, ancestry, cause of death, and body mass.

IDENTIFIED HUMAN SKELETAL COLLECTIONS							
COLLECTION	William M. Bass Forensic Skeletal Collection	Stanford Collection	Hamann-Todd Collection	Maxwell Museum Documented Skeletal Collection	Cape Town Documented Skeletal Collection	George Huntington Collection	J.C.B. Grant Collection
INSTITUTION	Department of Anthropology, The University of Tennessee	Department of Anthropology, University of Iowa	Cleveland Museum of Natural History	Maxwell Museum of Anthropology, University of New Mexico	Department of Anatomy and Cell Biology, University of Cape Town	Department of Anthropology, National Museum of Natural History	Department of Anthropology, University of Toronto
LOCATION	Knoxville, Tennessee, USA	Iowa City, Iowa, USA	Cleveland, Ohio	Albuquerque, New Mexico, USA	Cape Town, South Africa	Washington, DC, USA	Toronto, Ontario, Canada
SAMPLE	Consists of skeletal remains from over 100 forensic cases from the 1970s to the present	This collection is composed of around 1100 individuals that originally served as cadavers for anatomy classes at Stanford University Medical School.	consists of roughly 3100 modern human skeletons of both males and females of all ages and mainly of African and European ancestry.	Contains 257 individuals as of February 2008, although only 207 of those individuals are positively identified. Ages at death range from fetal to over 80 years.	The collection is derived from dissection cadavers used by the Department of Anatomy and Cell Biology between 1980 and 1996 and is of unknown size.	Comprised of over 3600 individuals with dates of death from 1892 to 1920.	Consists of 202 individuals who died and were received by the university between 1928 and the early 1950s. Most of the individuals are male and aged 40 years or older.
INFORMATION	Contains examples of a variety of types of trauma.	Birth dates range from the mid to late 1800s.		The amount of documentation varies depending on the source of the remains, but typically includes age, sex, and ancestry.	Documentation includes sex, ancestry, and age.	Age, sex, nationality, and cause of death are known. All individuals are either European immigrants or New York City residents. Many skeletons are incomplete due to their origin as dissection cadavers.	Demographic information consists of sex, age at death, and cause of death.

1.4) Aim of the present study

In bioarchaeology and forensic anthropology age and sex assessment of individuals from osteological feature is an important research theme.

The identified collections have a great relevance to test the development of reliable sex and age estimation techniques but we have to consider the limited number of osteological collections identified for age and sex used as a reference to test the degree of correspondence between estimated age and chronological age and to develop new methods for this purpose.

The collection of Bologna has never been used for this purpose because the information about the individuals have been cross checked only recently with the Bologna cemetery archives by members of the research group of the Laboratory of Bioarchaeology and Forensic Osteology. The high reliability of the collected information makes it one of the best European documented human collection.

In order to address the problem of reliability of methods for sex and age determination in human remains that have been highlighted by many authors (Saunders et al. 1992; Schmitt, 2004; Sakaue, 2006; Hens et al., 2008; Belcastro et al., 2008; Rivera - Sandoval, 2014), it has been decided to analyse the two European identified skeletal collections housed at the Universities of Bologna and Coimbra to test five methods for the age estimation in adults individuals (> 18 years) and two methods for the sex determination.

These methods are: synostosis of cranial sutures (Meindl and Lovejoy, 1985), morphological variations of the pubic symphysis (Suchey and Brooks, 1990), morphological variations of the ilium surface (Lovejoy et al., 1985), modifications of the sternal end of the fourth rib (Iscan et al., 1984, 1985, 1986), variation of the degree of sacral vertebral body fusion (Belcastro et al. 2008) for the age estimation and the index of sexualization of Acsadi and Nemeskeri (1970) and the Phenice method (Phenice, 1969) for the sex determination.

2) Materials and methods

The human skeletons used in this study belongs to the skeletal collection from the Certosa Cemetery of Bologna, Italy, housed in the department of Biology, Geology and Environmental Science of Alma Mater Studiorum University of Bologna (fig. 2.1a) and from the “Colecção Esqueletos Identificados” of Coimbra housed in the Departamento de Ciências da Vida, Universidade de Coimbra (Portugal) (fig. 2.1b).
In this study we tested five commonly used macroscopic methods to estimate age based on the observation of morphological changes of different districts: pubic symphysis (Suchey and Brooks 1990), auricular surface of the ileum (Lovejoy et al. 1985), costochondral end of the fourth rib (Iscan et al., 1984, 1985, 1986), suture closure in the skull (Meindl and Lovejoy 1985) and variation of the degree of sacral vertebral body fusion (Belcastro et al. 2008). Morphological variations in said skeletal districts are affected by a complex interaction of endogenous and exogenous factors, resulting in a wide inter and intra population variability. We also test the reliability of two macroscopic methods for sex determination: the index of sexualization of Acsadi and Nemeskeri (1970), and the Phenice method (Phenice, 1969).

Figure 2.1 Collection of Bologna a) and Coimbra b)

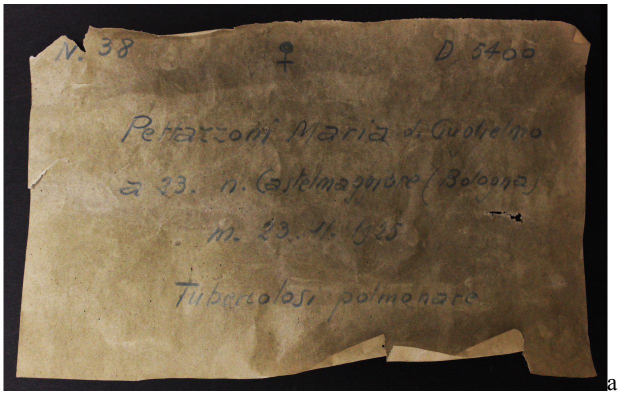
2.1) The sample

The study was carried out on two identified human skeletal samples belonging to the collections of Bologna and Coimbra. The individuals lacking information about sex and age were not included in the study. The collection of Bologna consists of identified skeletal remains of people exhumed from the Certosa Cemetery of Bologna. The collection was gradually accumulated by Prof. Fabio Frassetto founder of the Department and Museum of Anthropology of the University of Bologna and director from 1908 to 1947, and subsequently by Prof. Elsa Graffi Benassi, director till 1971. The individuals died in a period ranging from 1898 to 1944. The skeletons are collected in wooden boxes labeled with a number that follows the exhumation order. Males and females are numbered independently (male: 1-166; female 1-130). The same number labeling the box is copied using indian ink on all bones of all skeletons. Moreover, the symbol identifying the individual as male (♂) or female (♀) was drawn on the skull (fig. 2.2).

Figure 2.2 Skull of a male of the collection Bologna. Serial number, name and age of the individual are annotated on the right temporal bone.

A personal card reporting information about sex, age, birth-death place, death date, profession and death cause is associated to the majority of the individuals (fig. 2.3 a). In Spring 2015, a detailed check of the personal information regarding each individual from the archives of the Certosa cemetery of Bologna was concluded by the staff of the Laboratory of Bioarchaeology and Forensic osteology, in particulary by Dott.ssa Benedetta Bonfiglioli.

The Coimbra Identified Skeletal Collection is curated in the Anthropological Museum of the Department of Science and Technology of the University of Coimbra (Portugal). This collection was collected by Prof. Eusebio Tamagnini and consists of 505 skeletons dated to the first half of the 20th century (Rocha, 1995). The individuals of the collection died between 1910 and 1936, and were exhumed from the cemetery of Coimbra (Santos, 2000). For each individual, a record exists which provides cause of death and other medical and personal information. The information about each individual are reported in a book completed with data taken from different kind of documents as the cemetery record, the archive of Forensic Institute, and the archive of University of Coimbra (fig. 2.3 b). The individuals are conserved in wooden boxes.


N. 38 ♀ D. 5400

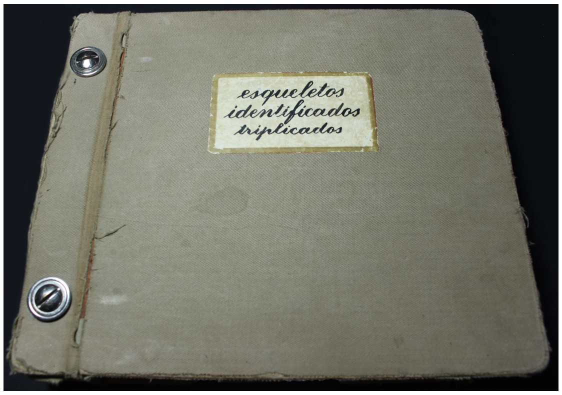
Pettazzoni Maria di Guglielmo

a. 23. n. Castelmaggiore (Bologna)

m. 23. 11. 1925

Tubercolosi polmonare

a)



b)

Figure 2.3 information about the individuals of the collection of Bologna a) and Coimbra b)

In this study we included 18 years or older individuals, described in table 2.1. The average age of the sample is 50,25 years old with a standard deviation of 18,47 in Coimbra sample and 50,33 years old with a standard deviation of 19,52 in Bologna sample.

Table 2.1 reports the average grade of subgroups.

	BOLOGNA	AGE	COIMBRA	AGE
MALE	135	18-91	120	21-86
FEMALE	112	18-87	122	18-91

Table 2.1. number of male and female with age range

	COIMBRA		BOLOGNA	
	MALE	FEMALE	MALE	FEMALE
AVERAGE	46,6	53,9	51,1	50,04
STANDARD DEVIATION	15,91	20,11	19,17	20,04

Table 2.2. average and standard deviation of the age of the sample studied

Table 2.3 report the distribution of the age of the samples and their standard deviation in a ten years range used in this study.

	COIMBRA			
	MALE		FEMALE	
age range	N.	Average grade (DS)	N.	Average grade (DS)
20-29	21	25,47 (+/- 3,06)	18	24,66 (+/- 3,03)
30-39	25	34, 52 (+/- 3,06)	19	35,05 (+/- 3,34)
40-49	23	44,65 (+/- 2,88)	15	44,2 (+/- 3,27)
50-59	24	54,17 (+/- 2,91)	21	54,14 (+/-2,95
60+	27	69,07 (+/- 6,85)	49	74,73 (+/- 8,44)

	BOLOGNA			
	MALE		FEMALE	
age range	N.	Average grade (DS)	N.	Average grade (DS)
20-29	27	23,5 (+/- 3,46)	23	24,43 (+/- 3,18)
30-39	18	34,9 (+/- 3,26)	21	34,3 (+/- 3,15)
40-49	16	44,7 (+/- 2,93)	12	44 (+/- 2,79)
50-59	24	55,2 (+/- 3,01)	14	54,43 (+/- 3,77)
60+	49	70,9 (+/- 7,06)	42	72,2 (+/- 7,58)

Table 2.3. distribution of the age of the samples and their standard deviation in a ten years range.

In this study we considered male and female samples separately and sixty years or older (60+) individual as a single group.

The 60+ individuals are represented in detail in chart 2.1 which compares the number of individuals of each group for each studied range.

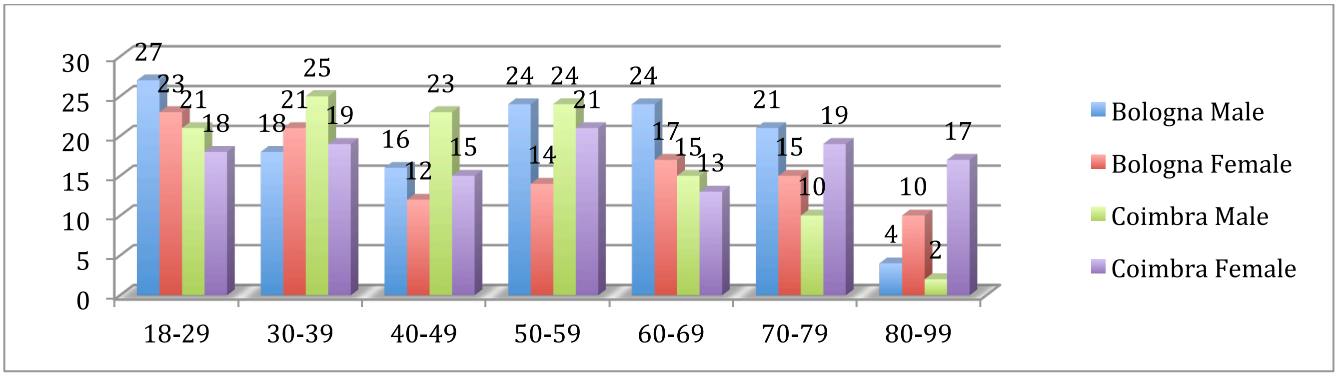


Chart 2.1. number of individual with range of 10 years

The total sample consists of 489 individuals who came from the collection of Bologna (247) as well as the collection of Coimbra (242) as described in table 2.4.

COLLECTION	M+F	M	F
BOLOGNA	247	135	112
COIMBRA	242	120	122
TOT.	489	255	234

Table 2.4. sample examined of each collections.

2.2) Methods

2.3) Methods tested for the age estimation

In this study we tested the reliability of selected macroscopic methods for age estimation and sex determination in adults on Italian and Portoguese samples. The skeletons were examined with no information about the individual history.

The method applied for the age estimation are:

- synostosis of cranial sutures (Meindl and Lovejoy, 1985);
- morphological variations of the pubic symphysis (Suchey and Brooks, 1990);
- morphological variations of the ileum surface (Lovejoy, et al. 1985);
- modifications of the sternal end of the fourth rib (Iscan et al., 1984, 1985, 1986);
- variation of the degree of sacral vertebral body fusion (Belcastro et al. 2008).

For methods based on the observation of the pelvis and the fourth rib, left and right side were assessed separately and analyzed both joined and separately. When right and left side were assigned to different stages, a mean value was assigned as estimated age of the individual to calculate the index of bias and inaccuracy.

- synostosis of cranial sutures (Meindl and Lovejoy, 1985)

The method used in this study was proposed by Meindl and Lovejoy (1985) and studied on 236 skulls of a sample of modern African Americans and European Americans from the Hamann-Todd Collection.

This method identifies 10 points in the skull (fig. 2.5) to evaluate the degree of obliteration of cranial sutures: 1 midlambdoid, 2 lambda, 3 obelion, 4 anterior sagittal, 5 bregma, 6 midcoronal, 7 pterion, 8 sphenofrontal, 9 inferior sphenotemporal, 10 superior sphenotemporal. The first seven of these sites are referred to as the vault system whereas the last three, when combined with pterion and midcoronal, are referred to as the lateral-anterior system.

A specific degree of closure was assigned to each site by the authors (table 2.5 fig. 2.4):

DEGREE	STATE	DESCRIPTION
0	open	There is no evidence of any ectocranial closure of the site
1	minimal closure	Some closure has occurred. This score in given for any minimal to moderate closure, i.e. from a single bony bridge across the suture to about 50% synostosis at the site;
2	significant closure	There is a marked degree of closure but some portion of the site is still not completely fused;
3	complete obliteration	The site is completely fused

Table 2.5 specific degree of closure assigned to each site (from Meindl and Lovejoy, 1985).

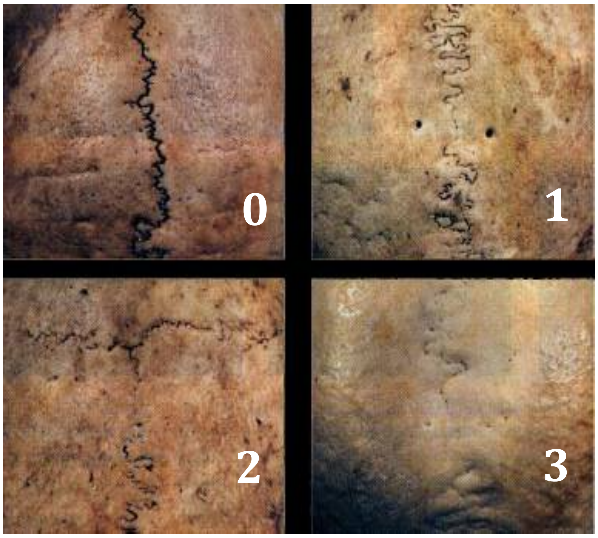


Figure 2.4 representation of specific degree of closure assigned to each site (modified from Cattaneo Grandi, 2004).

A grade of obliteration is assigned to each point. A composite score was calculated by summing the value given to each point considering the bilateral points just one time. In the case of different score for bilateral points, the higher value was considered.

The age estimated is given for two systems: the vault and the lateral-anterior system following the tables shown in fig. 2.6. The arithmetic mean of the two values was calculated as well. Whenever it was not possible to assign a score to every point due to taphonomic damage in the interested area, the final age estimate was obtained from the arithmetic mean of the average age associated to the degree of closure observed in each evaluated point by the authors.

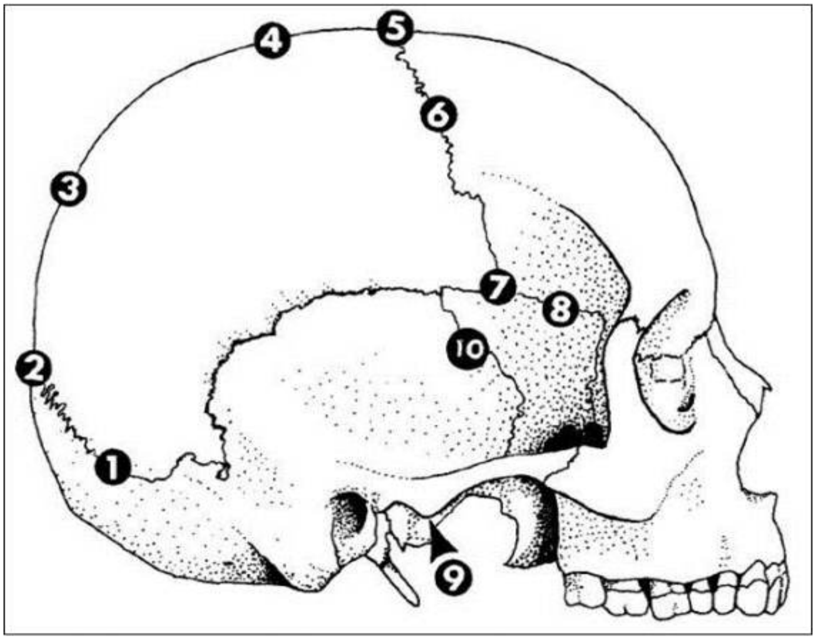


Figure. 2.5 ten points of the skull: 1 midlambdoid, 2 lambda, 3 obelion, 4 anterior sagittal, 5 bregma, 6 midcoronal, 7 pterion, 8 sphenofrontal, 9 inferiori sphenotemporal, 10 superior sphenotemporal (from Meindl and Lovejoy, 1985).

TABLE 6. Determination of age based on ectocranial lateral-anterior suture closure

Composite score	No.	Mean age	Standard dev.	Mean dev.	Inter-decile range	Range
0 (Open)	42				–43	–50
1	18	32.0	8.3	6.7	21–42	19–48
2	18	36.2	6.2	4.8	29–44	25–49
3, 4, 5	56	41.1	10.0	8.3	28–52	23–68
6	17	43.4	10.7	8.5	30–54	23–63
7, 8	31	45.5	8.9	7.4	35–57	32–65
9, 10	29	51.9	12.5	10.2	39–69	33–76
11, 12, 13, 14	24	56.2	8.5	6.3	49–65	34–68
15 (Closed)	1					
	236					

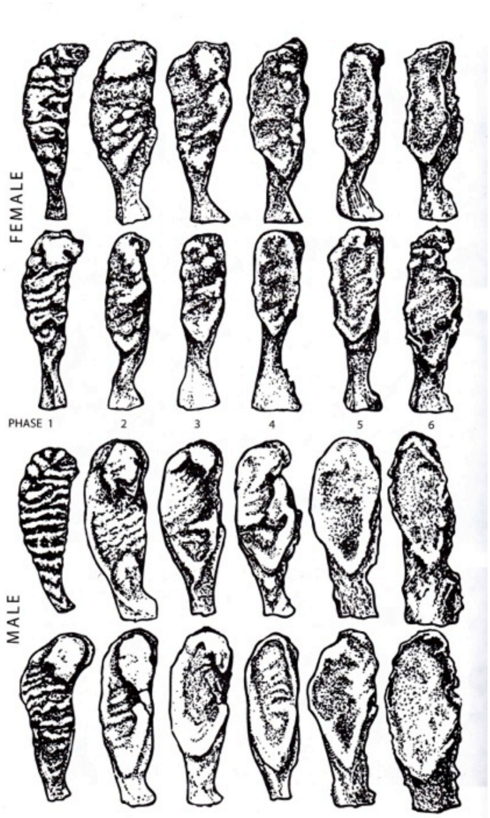
TABLE 7. Determination of age based on ectocranial vault sutures

Composite score	No.	Mean age	Standard dev.	Mean dev.	Inter-decile range	Range
0 (open)	24				–35	–49
1, 2	12	30.5	9.6	7.4	19–44	18–45
3, 4, 5, 6	30	34.7	7.8	6.4	23–45	22–48
7, 8, 9, 10, 11	50	39.4	9.1	7.2	28–44	24–60
12, 13, 14, 15	50	45.2	12.6	10.3	31–65	24–75
16, 17, 18	31	48.8	10.5	8.3	35–60	30–71
19, 20	26	51.5	12.6	9.8	34–63	23–76
21 (Closed)	13				43–	40–
	236					

Figure 2.6 age estimates based on ectocranial suture closure (from Meindl and Lovejoy, 1985).

- morphological variations at the pubic symphysis (Brooks and Suchey, 1990)

Brooks and Suchey (1990) offers a revision of a method for determining the age from the pubic symphysis, tested on recent autopsied remains from the Los Angeles County Coroner. Suchey collected a 1225 well-documented sample of pubic bones in a period of three years (1977-1979) for this research. The age of these samples was derived from death and /or birth certificates. The sample consists of 739 male and 273 female individuals ranging from age 14 to 99 years. The legal archive provided information on race, occupation and socio-economic class. For the female group the information also added the number of children deliveries. The sample was considered representative of the general population in terms of socio-economic class (Arenosa and Suchey, 1987). According to age-related osteological features, which are common to both male and female, the pubic symphysis face is classified into six phases. Each phase presents a standard deviation given by sex (Brooks and Suchey, 1990). The observer has to consider the pubic symphysis to assess which of the six phases best represents the morphological aspect. The overall phases have a range from 15 to 87 years (fig. 2.7).



2.7a)

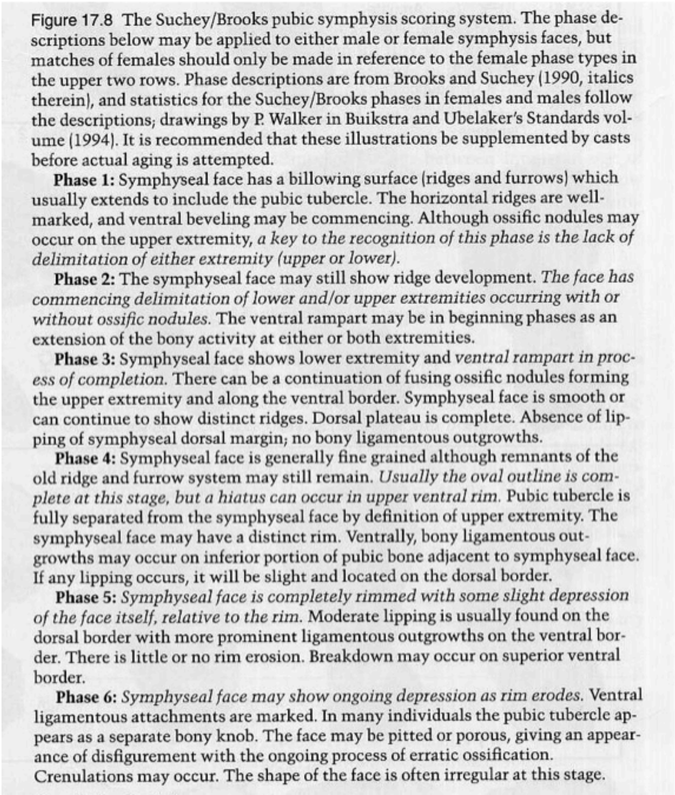


Figure 17.8 The Suchey/Brooks pubic symphysis scoring system. The phase descriptions below may be applied to either male or female symphysis faces, but matches of females should only be made in reference to the female phase types in the upper two rows. Phase descriptions are from Brooks and Suchey (1990, italics therein), and statistics for the Suchey/Brooks phases in females and males follow the descriptions; drawings by P. Walker in Buikstra and Ubelaker's Standards volume (1994). It is recommended that these illustrations be supplemented by casts before actual aging is attempted.

Phase 1: Symphyseal face has a billowing surface (ridges and furrows) which usually extends to include the pubic tubercle. The horizontal ridges are well-marked, and ventral beveling may be commencing. Although ossific nodules may occur on the upper extremity, *a key to the recognition of this phase is the lack of delimitation of either extremity (upper or lower).*

Phase 2: The symphyseal face may still show ridge development. *The face has commencing delimitation of lower and/or upper extremities occurring with or without ossific nodules.* The ventral rampart may be in beginning phases as an extension of the bony activity at either or both extremities.

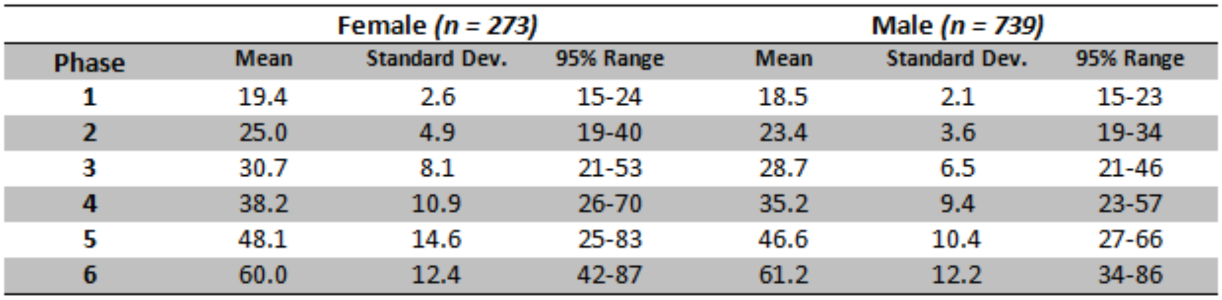
Phase 3: Symphyseal face shows lower extremity and *ventral rampart in process of completion.* There can be a continuation of fusing ossific nodules forming the upper extremity and along the ventral border. Symphyseal face is smooth or can continue to show distinct ridges. Dorsal plateau is complete. Absence of lipping of symphyseal dorsal margin; no bony ligamentous outgrowths.

Phase 4: Symphyseal face is generally fine grained although remnants of the old ridge and furrow system may still remain. *Usually the oval outline is complete at this stage, but a hiatus can occur in upper ventral rim.* Pubic tubercle is fully separated from the symphyseal face by definition of upper extremity. The symphyseal face may have a distinct rim. Ventrally, bony ligamentous outgrowths may occur on inferior portion of pubic bone adjacent to symphyseal face. If any lipping occurs, it will be slight and located on the dorsal border.

Phase 5: *Symphyseal face is completely rimmed with some slight depression of the face itself, relative to the rim.* Moderate lipping is usually found on the dorsal border with more prominent ligamentous outgrowths on the ventral border. There is little or no rim erosion. Breakdown may occur on superior ventral border.

Phase 6: *Symphyseal face may show ongoing depression as rim erodes.* Ventral ligamentous attachments are marked. In many individuals the pubic tubercle appears as a separate bony knob. The face may be pitted or porous, giving an appearance of disfigurement with the ongoing process of erratic ossification. Crenulations may occur. The shape of the face is often irregular at this stage.

2.7b)



	Female *(n = 273)*			Male *(n = 739)*		
Phase	**Mean**	**Standard Dev.**	**95% Range**	**Mean**	**Standard Dev.**	**95% Range**
1	19.4	2.6	15-24	18.5	2.1	15-23
2	25.0	4.9	19-40	23.4	3.6	19-34
3	30.7	8.1	21-53	28.7	6.5	21-46
4	38.2	10.9	26-70	35.2	9.4	23-57
5	48.1	14.6	25-83	46.6	10.4	27-66
6	60.0	12.4	42-87	61.2	12.2	34-86

2.7c)

Figure 2.7 a) graphic representation of the various stages; b) description of the different phases; c) range and average age of the different stages, from Buikstra and Ubelaker, 1994.

In this work we considered a confidence interval of 95 indicated by the authors and 67% proposed in Cattaneo Grandi (2004) to establish the match of the known age in the range of the method. The wide range shown in the picture 3c for each phases must be taken into consideration in the discussion of this work.

- auricular surface (Lovejoy et al., 1985)

A method to estimate the age from the auricular surface was developed by Lovejoy and his collaborators in 1985. In order to develop this method the authors used more than 250 individuals from Libben collection (dating from late first millennium), and a sample of roughly 500 modern and contemporary African Americans and European Americans from the Hamann-Todd collection and 14 forensic cases (Cuyahoga Country Coroner's Office). Lovejoy et al. elaborated eight phases with a 5-year sex-independent step, from 20 to 60 years old (figure 2.8-2.9). Each phase considers surface texture, transverse organization, porosity, and arthritic changes.

Observed coxal bones have to be associated with the phase best representing the morphological appearances in order to assess age.

A short description of the morphological change described from the authors for each phase follows:

PHASE	AGE RANGE	DESCRIPTION
1	20-24	billowing and very fine granularity.
2	25-29	reduction of billowing, but retention of youthful appearance
3	30-34	general loss of billowing, replacement by striae, and distinct coarsening of granularity
4	35-39	uniform coarse granularity
5	40-44	transition from coarse granularity to dense surface; this may take part over islands of the surface of one or both faces.
6	45-49	completion of densification with complete loss of granularity
7	50-59	dense irregular surface of rugged topography and moderate to marked activity in periauricular areas
8	60+	breakdown with marginal lipping, macroporosity, increased irregularity, and marked activity in periauricular areas

Table 2.6. description of the morphological change of the auricular surface for each phase (from Lovejoy et al., 1985).

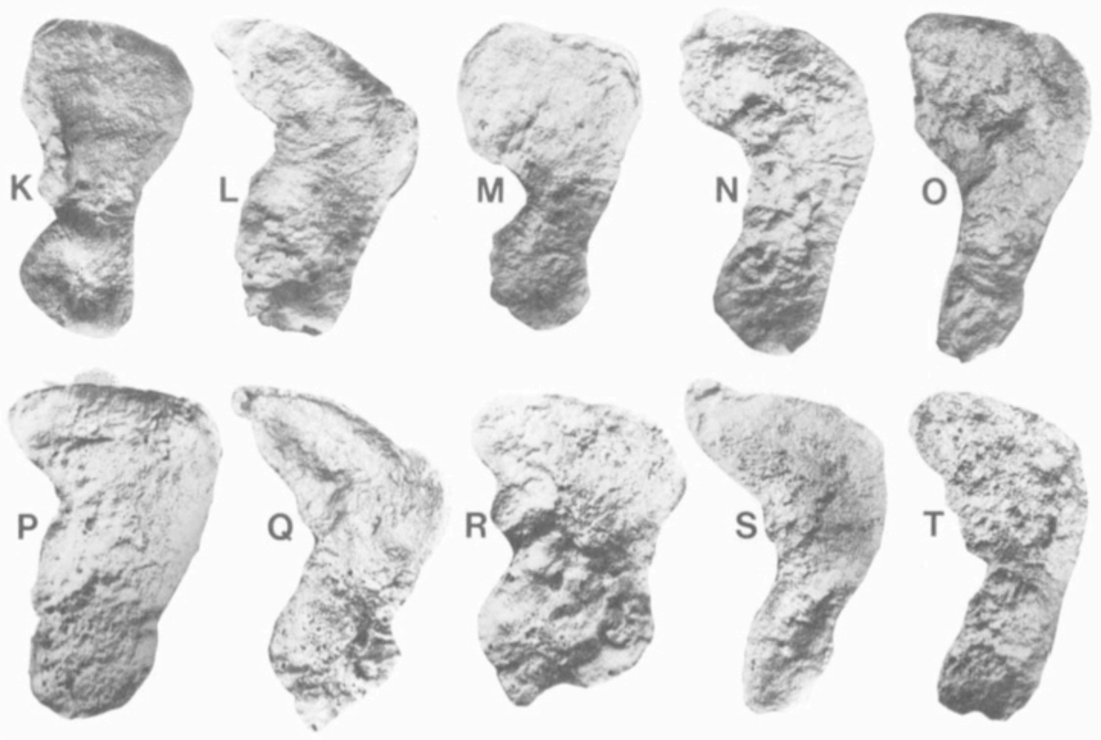


Figure 2.8 modal changes in the auricular surface with age: third and fourth decades (A-J) (Lovejoy et al., 1985)

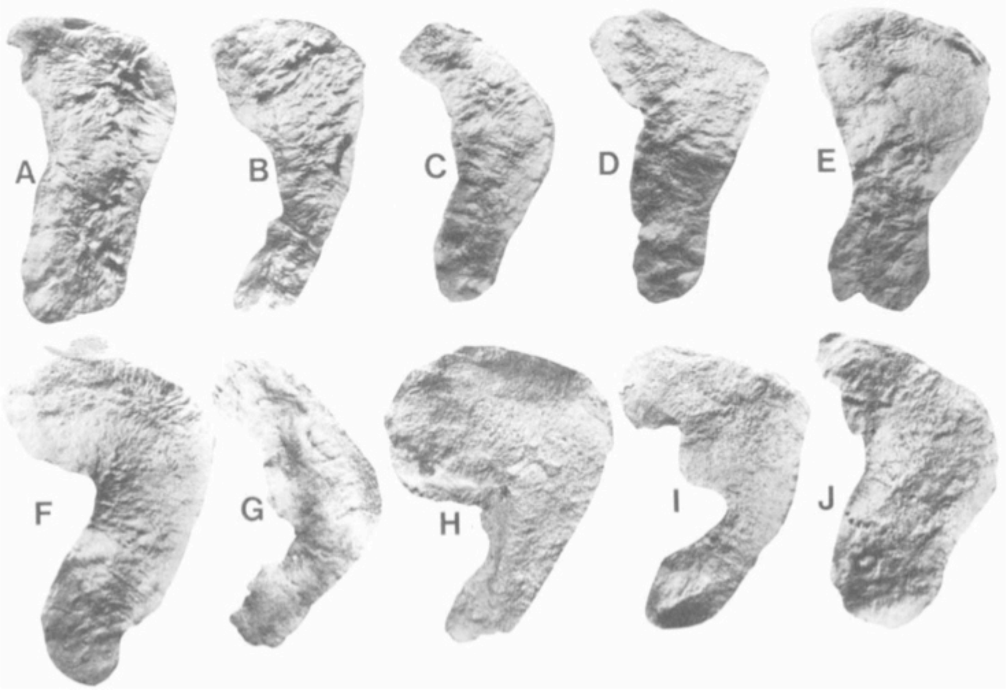


Figure 2.9 modal changes in the auricular surface with age: fifth and sixth decade (K-T) (Lovejoy et al., 1985).

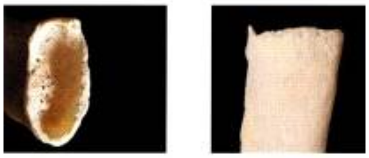
- modifications of the sternal end of the fourth rib (Iscan et al., 1984, 1985, 1986)

The method of Iscan's rib is a reliable indicator of age at death in the adult skeleton (Iscan et.al, 1984, Iscan et.al. 1985). Iscan et al. developed a method by using sternal end of fourth rib in 1984 on 118 white males (autopsied at the Broward County Medical Examiner's Office) (Iscan et.al, 1984a) they also developed phase analysis method on 86 white females (Iscan et al. 1985). The morphological changes of the fourth rib due to the senescence process depend on the sex and on the different geographical group (Iscan et al., 1984; Iscan et al., 1985; Iscan 1987; Iscan and Loth 1986; Loth et. al 1994). The choice of the fourth rib is simply conventional (Cattaneo, Grandi 2004). The method describes the junction between the anterior portion of the fourth rib and the cartilage innesting in the sternum: this surface presents a concavity with a regular margin in the young individuals, whereas in the older ones the concavity is deeper with thin and irregular margins caused by senescent artrosic osteophytes. The authors identified nine phases with slight differences between the two sexes (fig. 2.10).

Phase 0
M: <16 y.
F: <13 y.

Phase 1
M: 17-19 y.
F: 14-15 y.

Phase 2
M: 20-23 y.
F: 16-19y.



Phase 3
M: 24-28 y.
F: 20-24 y.

Phase 4
M: 26-32 y.
F: 24-32 y.

Phase 5
M: 33-42 y.
F: 33-46 y.

Phase 6
M: 43-55 y.
F: 43-58 y.

Phase 7
M: 54-64 y.
F: 59-71 y.

Phase 8
M: >65 y.
F: >70 y.

Figure 2.10 different phases of the sternal end of the fourth rib (from Cattaneo, Grandi 2004).

- variations of the degree of sacral vertebral body fusion (SVF) (Belcastro et al. 2008)

The study was conduced on 904 adult sacra: 418 sacra from the Sassari collection (SS; Sardinia, Italy), part of the Frassetto collections (Museum of Anthropology, University of Bologna, Italy) (Facchini et al., 2006), and 418 are from the Colecção Esqueletos Identificados (CMB; Museum of Anthropology, University of Coimbra, Portugal) (Rocha, 1995)).

The autor examined the degree of fusion of the sacral vertebral bodies (SVF), observing the ventral face of the sacrum. The autor identified four degree of fusion described in table 2.7. The score of the degree of body fusion at each site were represent in figure 2.11.

DEGREE	DESCRIPTION
0	absence of fusion
1	less than 50% fusion
2	more than 50% fusion
3	complete fusion

Table 2.7. four degree of fusione with respective description (Belcastro et al. 2008).

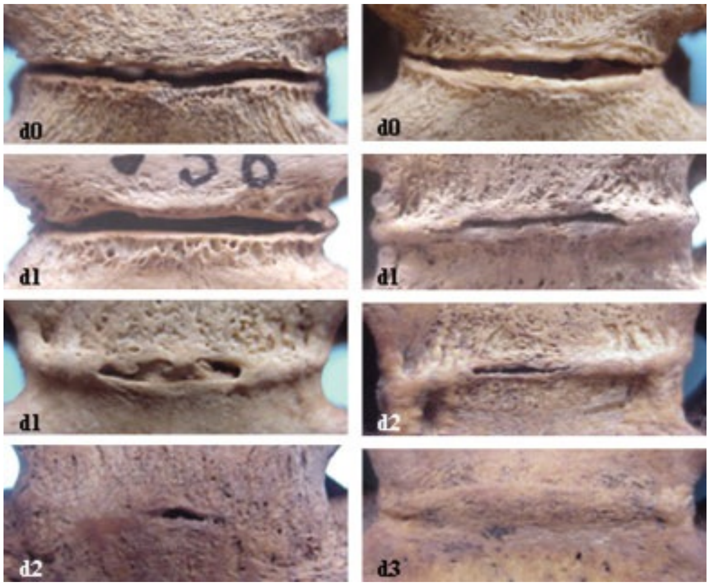


Figure 2.11 Standardized scoring of the Degree of SVF. d0=Degree 0, d1=Degree 1, d2=Degree 2, d3=Degree 3. The photographed material belongs to the SS collection (from Belcastro et al. 2008).

For each sacrum we also calculated a total fusion score, representing the mean degree of fusion of the entire bone (total score), a fusion score for the upper half (upper score) and a fusion score for the lower half (lower score). The fusion score is a number between 0 and 3.

- total score = $(S_{1–2} + S2_{–3} + S_{3–4} + S_{4–5})/4$
- upper score = $(S_{1–2} + S_{2–3})/2$
- lower score = $(S_{3–4} + S_{4–5})/2$

The pattern is a synthetic representation of the degrees of fusion of the four sites of each sacrum, illustrating the association between the stages of fusion of the different sites.

2.4) Methods tested for the sex determination

The methods for sex attribution are:

- the index of sexualization of Acsadi and Nemeskeri (1970), for the skull and pelvis;
- the Phenice method for the pelvis (Phenice, 1969).

- the index of sexualization of Acsadi and Nemeskeri (1970)

The index of sexualization (IS) elaborated by the authors considers 10 points of the cranium (fig. 2.14), 3 of the jaw (facies, mentum, angulus mandibulae) and 11 points of the pelvis (sulcus preauricularis, incisura ischiatica major, angulus pubis, arcus compositus, os coxae, foramen obturatum, corpus ossis ischia, crista iliaca, fossa iliaca, pelvis major, pelvis minor). The observer assigns a numerical value, ranging from -2 (hyperfemmine) to +2 (hypermasculine) to each diagnostic feature. The following formula based on the weighted average indicates the attributed sex:

$$IS = \sum (\text{score x weight}) / \sum \text{weight}$$

A positive IS value identifies a male individual, while a negative IS value identifies a female individual. A IS score equals or approaching to zero, must be regarded as uncertain sex (Ascadi and Nemeskeri, 1970).

Figure 2.12 female and male pelvis (from White & Folkens 2005)

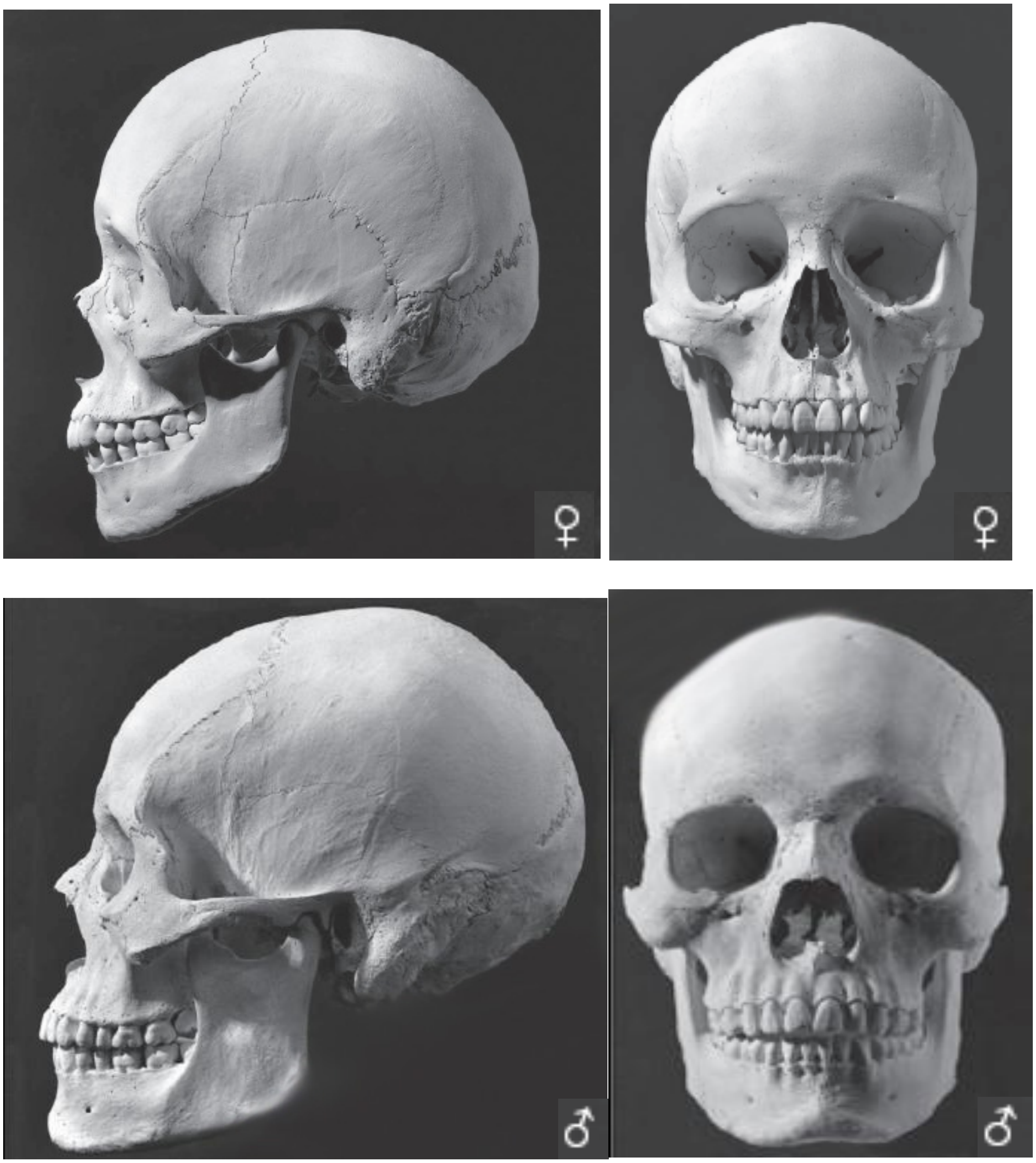
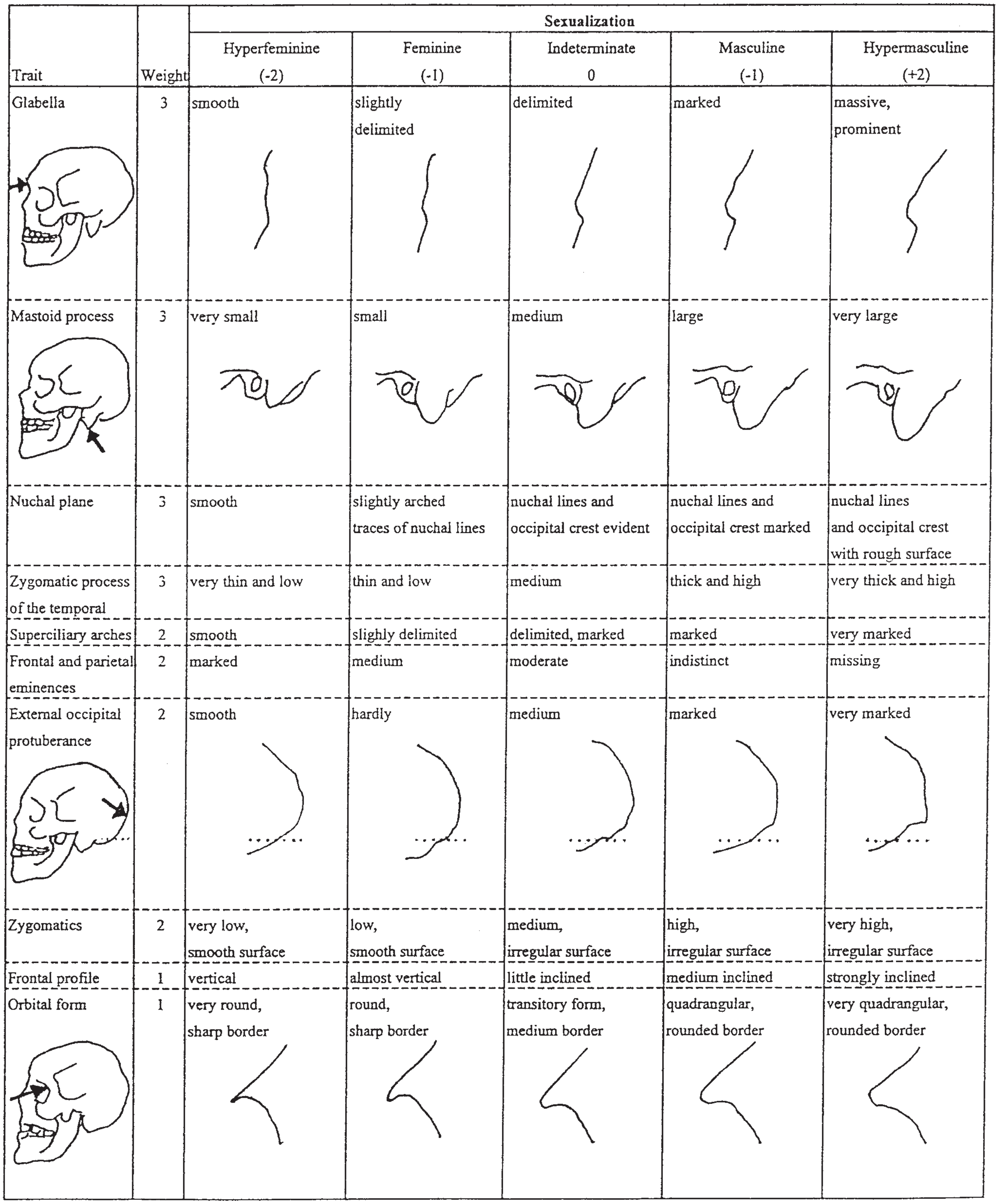


Figure 2.13 female and male skull (from White & Folkens 2005)



		Sexualization				
Trait	Weight	Hyperfeminine (-2)	Feminine (-1)	Indeterminate 0	Masculine (-1)	Hypermasculine (+2)
Glabella	3	smooth	slightly delimited	delimited	marked	massive, prominent
Mastoid process	3	very small	small	medium	large	very large
Nuchal plane	3	smooth	slightly arched traces of nuchal lines	nuchal lines and occipital crest evident	nuchal lines and occipital crest marked	nuchal lines and occipital crest with rough surface
Zygomatic process of the temporal	3	very thin and low	thin and low	medium	thick and high	very thick and high
Superciliary arches	2	smooth	slighly delimited	delimited, marked	marked	very marked
Frontal and parietal eminences	2	marked	medium	moderate	indistinct	missing
External occipital protuberance	2	smooth	hardly	medium	marked	very marked
Zygomatics	2	very low, smooth surface	low, smooth surface	medium, irregular surface	high, irregular surface	very high, irregular surface
Frontal profile	1	vertical	almost vertical	little inclined	medium inclined	strongly inclined
Orbital form	1	very round, sharp border	round, sharp border	transitory form, medium border	quadrangular, rounded border	very quadrangular, rounded border

Figure 2.14 Character traits for sex determination of the cranium (Walrath et al. 2004, modified from Ferembach et al. (1980) and Buikstra et al. (1994)).

- the Phenice method (1969)

Phenice recognized morphological features of the pubic bone useful in sex determination in 1969. The relative easiness of application and the high degree of accuracy made this method one of the widest used in the human osteological analysis (Bass, 1971; Steward, 1979; Ubelaker, 1978; Ortner and Putschar, 1981; Skinner and Lazenby, 1983). The author analyzed 275 individuals of know sex of European and African ancestry. The method allows the diagnosis of sex with a precision of 96% (Phenice, 1969). It also allows a diagnosis even though the coxal bone is not complete because it takes into consideration three morphological sex indicators of the pubic region of the os coxae described in table 2.8 and figure 2.15:

SECTION OF THE PUBIS	DESCRIPTION
Ventral Arc	"A slightly elevated ridge of bone which extends from the pubic crest and arcs inferiorly across the ventral surface to the lateral most extension of the sub-pubic concavity where it blends with the medial border of the ischio-pubic ramus."
Subpubic Concavity	"A lateral recurve in the ischio-pubic ramus a short distance below the lower margin of the pubic symphysis."
Ischiopubic Ramus Ridge	"A ridge on the ischio-pubic ramus immediately below the symphyseal surface."

Table 2.8. description of the site studied by Phenice 1969.

Following McLaughlin and Bruce (1990) it was established a diagnosis to each section - male (M), female (F) or inconclusive (0) - to the two sides of the pelvis, in the case of ambiguity (e.g. M, F, 0 or 0, 0, M or 0, 0, F) an inconclusive sex value (0) was attributed. The analysis of this information esteems the sex of the individual.

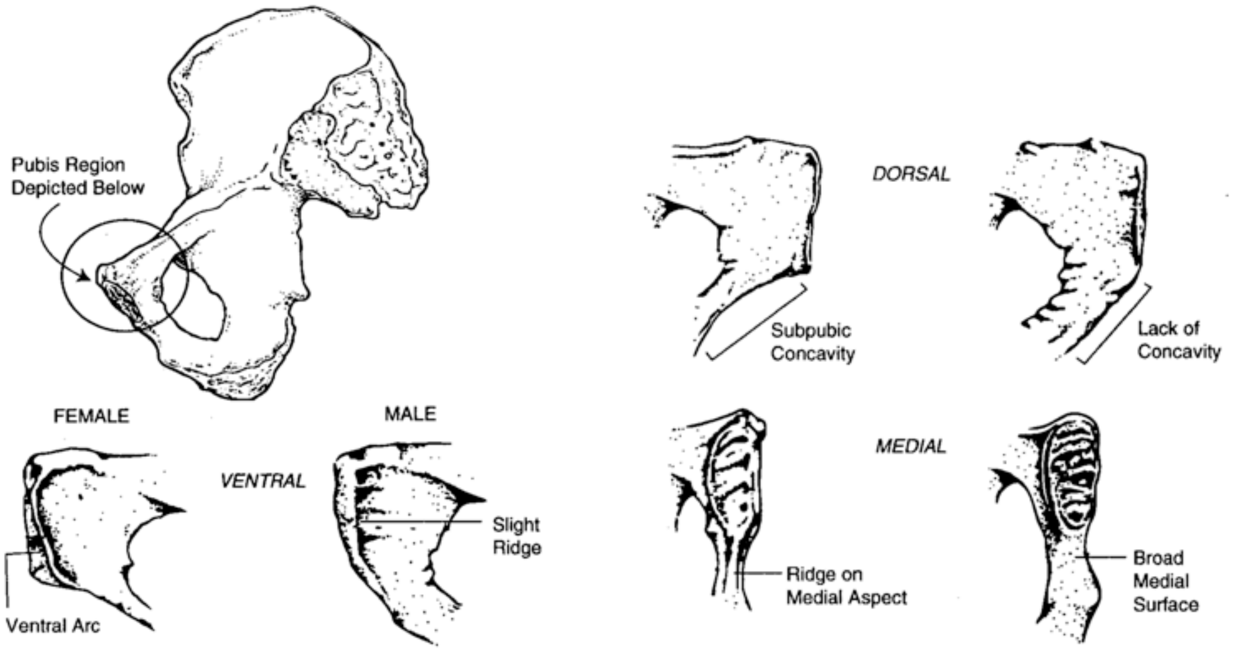


Figure 2.15 ventral arc, subpubic concavity and ischiopubic ramus ridge (from Buikstra Ubelaker, 1994).

2.5) Data analysis

We tested the reliability for the age estimation by using the standard measures of bias and inaccuracy. The index of bias is the mean over- or under- prediction, i.e., $\sum$ (estimated age-actual age) ⁄ n; whereas the index of inaccuracy is the average absolute error of age estimation without the reference to over- or under-prediction, i.e., $\sum$|estimated age-actual age| ⁄ n (Murray and Murray, 1991; Bedford, 1993; Schmitt, 2004; Hens, 2008). To calculate these indices for the methods that give an age range (variations of the pubic symphysis (Suchey and Brooks, 1990), morphological variations of the ilium surface (Lovejoy, et al. 1985) and modifications of the sternal end of the fourth rib (Iscan et al., 1984, 1985, 1986)) we considered the mean value indicated by the authors for the different morphological phases. For the aforementioned methods we considered the age range proposed by the authors to establish the match/mismatch with the known age. The same analysis was not carried out on the values obtained from the cranial suture closure method as, due to the considerable wideness of the age ranges, the information was deemed not relevant.

We used the free software R 2.9.2 (www.cran.r-project.org) to perform Spearman's rank correlation test and the respective significance. We considered the correlation statistically significant when the P value was lower than 0,05. RHO ranged between +1, positive perfect correlation, to -1, negative perfect correlation; 0 indicate absence of correlation.

CHAPTER 3: Results

For a clearer and simpler explanation, in the following chapter all the methods will be named without the repetition of the biblography.

3.1) Age estimation

- correlation between known and estimated age (Spearman's rho) for each method

The correlation between known and estimated age by different methods is calculated with the Spearman's coefficient. The results of the coefficient are high and significant except for the method of the 4th rib. The values of the method in both female samples (Bologna and Coimbra) are not reliable for too many ties.

	BOLOGNA						COIMBRA					
	M			F			M			F		
METHOD	N.	RHO	P-VALUE	N.	RHO	P-VALUE	N.	RHO	P-VALUE	N.	RHO	P-VALUE
CRANIAL SUTURE CLOSURE	134	0,933	0,0007	101	0,733	0,0311	120	0,857	0,0107	122	0,916	0,001
PUBIC SINPHYSIS	110	0,963	$<2,2^{-16}$	81	0,915	0,0004	102	0,983	$4,96^{-05}$	109	1	$<2,2^{-16}$
AURICULAR SURFACE	122	0,983	$4,96^{-0,5}$	110	0,966	0,0001	113	0,976	0,0003	98	0,933	0,0007
4° RIB	13	1	0,0027	32	0,61	0,0461	33	0,95	0,0003	28	0,765	0,009

Table 3.1. correlation between the know and estimated age for each method tested

- positive match between the known age and the range identified by the methods of pubic synphysis, auricular surface and 4th rib

Table 3.2 shows the percentages of positive match of the pubic symphysis, auricular surface and 4th rib in all of the samples. The pubic symphysis is the method with the highest percentage of individuals included in the age range identified by the method while the method that performed worst has been the auricular surface method.

	Pubic symphysis		Auricular surface	4° rib
	95%	67%		
% (n/tot)	91% (364/401)	67% (269/401)	39% (181/463)	50% (66/132)

Table 3.2. individual with positive match for pubic symphysis, auricular surface and fouth rib in the total sample (Bologna+Coimbra; n= number of individuals with positive match, tot= number of observed individuals).

Table 3.3 show the percentages of positive match of the pubic symphysis, auricular surface and 4th rib in the sample of Bologna (male+female) and Coimbra (male+female), in the total male sample (Bologna+Coimbra) and in the total female sample (Bologna+Coimbra). The pubic symphysis is the method with the highest percentage of individuals included in the age range identified by the method in all the subsamples. In the sample of Bologna the method with the highest percentange of mismatches is the one based on the analysis of the 4th rib, while in the sample of Coimbra the auricular surface method has the poorest performance. When analyzing the sample according to sex, we find that in the male sample the worst performing method was the auricular surface while in the female sample it was the fourth rib. However, when analyzing the results for the fourth rib method, the considerably lower sample size must be taken into account.

	BOLOGNA			
	Pubic symphysis		Auricular surface	4° rib
	95%	67%		
BO (M+F)	88% (167/190)	61% (115/190)	37% (86/232)	36% (24/66)
CO (M+F)	93% (197/211)	73% (154/211)	41% (95/231)	64% (42/66)
M (BO+CO)	86% (182/212)	65% (138/212)	32% (76/235)	59% (39/66)
F (BO+CO)	96% (182/189)	69% (131/191)	54% (105/228)	41% (27/66)

Table 3.3. individual with positive match (%, n/N) for pubic symphysis, auricular surface and fouth rib in the sample of Bologna (male+female), Coimbra (male+female), male (Bolona+Coimbra), female (Bolona+Coimbra).

In table 3.4 is reported, in detail, the percentage of individuals correctly classified by the different methods in the subgroups studied: males and females of both collections and left and right side of the district were analyzed separately.

We will use abbreviations to refer to the different samples: MB = males Bologna, FB = females Bologna, MC = males Coimbra, FC = females Coimbra.

	PUBIC SYMPHISYS				AURICULAR SURFACE		FOURTH RIB	
	RIGHT 95%	LEFT 95%	RIGHT 67%	LEFT 67%	RIGHT	LEFT	RIGHT	LEFT
MB	81% (83/102)	77% (65/84)	57% (58/102)	56% (47/84)	23% (27/119)	17% (19/110)	39% (7/18)	46% (12/26)
FB	98% (65/66)	96% (66/69)	64% (42/66)	64% (44/79)	42% (44/106)	42% (45/106)	32% (9/28)	30% (8/27)
TOT B	88% (148/168)	86% (131/153)	60% (100/168)	56% (91/163)	32% (71/225)	30% (64/216)	35% (16/46)	38% (20/53)
MC	92% (87/95)	90% (85/94)	72% (68/95)	74% (70/94)	28% (30/106)	34% (37/109)	76% (16/21)	62% (18/29)
FC	96% (99/103)	96% (101/105)	72% (74/103)	7% (74/105)	37% (42/114)	35% (40/114)	50% (14/28)	44% (12/27)
TOT C	94% (186/198)	93% (186/199)	72% (142/198)	72% (144/199)	33% (72/220)	35% (77/223)	61% (30/49)	54% (30/56)
TOT B+C	92% (334/365)	90% (317/352)	73% (242/365)	73% (235/352)	32% (143/445)	32% (141/439)	48% (46/95)	46% (50/109)

Table 3.4. % Of individuals (n/N) who falls in the range estimated for each method. MB: male Bologna, MF female Bologna, MC male Coimbra, FC female Coimbra, TOT B total Bologna , TOT C total Coimbra, TOT total sample.

The method of the pubic symphysis shows positive matches over 90% in the sample of Coimbra in the confidence interval of 95%; the female samples in both collections show higher percentages of positive matche than the male samples, and the right pubic side shows slightly higher percentage of positive matches for the considered confidence interval.

The auricular surface shows the maximum percentage of positive match in the female sample of Bologna (42 %).

The male samples show higher discrepancies between left and right side of the pelvis. The method of the fourth rib shows the highest percentage of positive match in the male sample of Coimbra (76 %).

Table 3.5 shows the results divided into 10 years age ranges. The method of the pubic symphysis show a positive match of 100 % in the confidence interval of 95 % in all groups from 30 to 49 years, 100% of positive match is also in the range 50 to 59 MB and 20 to 29 MC. The method is remarkably reliable in females of Coimbra: we found positive match of 100% in the range 18 to 68 years. There are no particular differences between the right side and the left side in the two collections. The method of auricular surface does not shows good results in any group, the best performance is in the FB in the range from 80 to 87 with 60% of positive matches.

	Pubic symphysis				Auricular surface		4° rib	
	R 95%	L 95%	R 67%	L 67%	R	L	R	L
AGE RANGE	**MALE BOLOGNA**							
18-29	94% (17/18)	83% (10/12)	83% (15/18)	75% (9/12)	16% (4/25)	13% (3/23)	50% (2/4)	63% (5/8)
30-39	100% (13/13)	100% (10/10)	77% (10/13)	100% (10/10)	35% (6/17)	25% (4/16)	50% (2/4)	50% (2/4)
40-49	100% (13/13)	100% (11/11)	69% (9/13)	64% (7/11)	43% (7/16)	20% (3/15)	50% (1/2)	50% (1/2)
50-59	100% (18/18)	94% (15/16)	67% (12/18)	75% (12/16)	13% (3/22)	10% (2/20)	- (0/2)	- (0/4)
60-69	78% (18/23)	75% (15/20)	52% (12/23)	45% (9/20)	5% (1/22)	5% (1/21)	25% (1/4)	60% (3/5)
70-79	24% (4/17)	27% (4/15)	- (0/17)	- (0/15)	35% (6/17)	40% (6/15)	50% (1/2)	33% (1/3)
80-87	-	-	-	-	-	-	-	-
	FEMALE BOLOGNA							
18-29	100% (14/14)	94% (15/16)	86% (12/14)	81% (13/16)	18% (4/22)	18% (4/22)	33% (3/9)	27% (3/11)
30-39	100% (17/17)	100% (14/14)	88% (15/17)	93% (13/14)	71% (15/21)	55% (11/20)	16% (1/6))	20% (1/5)
40-49	100% (7/7)	100% (9/9)	86% (6/7)	89% (8/9)	40% (4/10)	41% (5/12)	100% (3/3)	100% (3/3)
50-59	100% (7/7)	88% (7/8)	86% (6/7)	88% (7/8)	57% (8/14)	69% (9/13)	28% (2/7)	- (0/3)

60-69	90% (9/10)	89% (8/9)	30% (3/10)	33% (3/9)	18% (3/16)	26% (4/15)	- (0/1)	50% (1/2)
70-79	100% (7/7)	100% (7/7)	- (0/7)	- (0/7)	38% (5/13)	42% (6/14)	- (0/2)	- (0/3)
80-87	100% (4/4)	100% (6/6)	- (0/4)	- (0/6)	50% (5/10)	60% (6/10)	-	-
	MALE COIMBRA							
18-29	100% (14/14)	100% (14/14)	100% (14/14)	100% (14/14)	6% (1/17)	10% (2/20)	100% (3/3)	100% (1/1)
30-39	100% (18/18)	100% (18/18)	89% (16/18)	94% (17/18)	48% (11/23)	65% (15/23)	50% (1/2)	60% (3/5)
40-49	100% (20/20)	100% (19/19)	90% (18/20)	94% (18/19)	39% (9/23)	33% (7/21)	100% (5/5)	57% (4/7)
50-59	95% (18/19)	95% (18/19)	63% (12/19)	63% (12/19)	36% (8/22)	36% (8/22)	80% (4/5)	71% (5/7)
60-69	79% (11/14)	71% (10/14)	36% (5/14)	43% (6/14)	- (0/13)	7% (1/15)	50% (1/2)	66% (4/6)
70-78	67% (6/9)	60% (6/10)	30% (3/10)	30% (3/10)	13% (1/8)	50% (4/8)	50% (2/4)	33% (1/3)
80-87	-	-	-	-	-	-	-	-
	FEMALE COIMBRA							
18-29	100% (13/13)	100% (14/14)	85% (11/13)	85% (12/14)	7% (1/15)	- (0/15)	33% (2/6)	33% (2/6)
30-39	100% (17/17)	100% (16/16)	100% (17/17)	93% (15/16)	61% (11/18)	68% (13/19)	75% (3/4)	66% (2/3)
40-49	100% (12/12)	100% (12/12)	100% (12/12)	100% (12/12)	40% (6/15)	35% (5/14)	25% (1/4)	25% (1/4)
50-59	100% (17/17)	100% (18/18)	100% (17/17)	100% (18/18)	48% (10/21)	35% (7/20)	100% (1/1)	50% (1/2)
60-69	100% (12/12)	100% (12/12)	100% (12/12)	100% (12/12)	31% (4/13)	25% (3/12)	100% (3/3)	100% (1/1)
70-78	100% (18/18)	100% (18/18)	28% (5/18)	27% (5/18)	18% (3/17)	16% (3/18)	33% (2/6)	42% (3/7)
80-87	71% (10/14)	73% (11/15)	- (0/14)	- (0/15)	47% (7/15)	56% (9/16)	50% (2/4)	50% (2/4)

Table 3.5. % of individuals (n/N) of the collection of Bologna and Coimbra for which the known age falls in the estimated range, by 10 years.

- bias and inaccuracy of tested methods

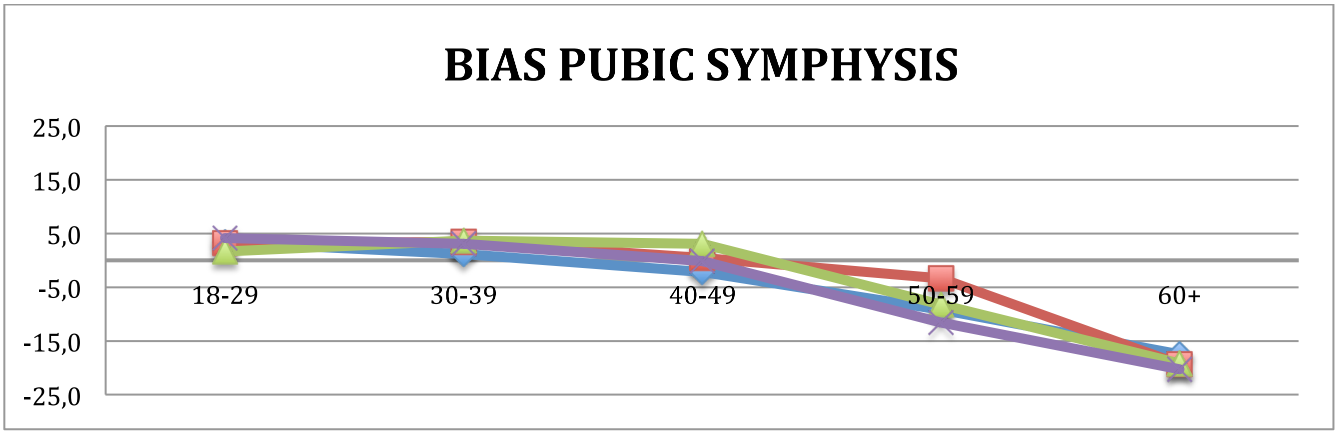
In tables 3.6 are reported the results of the index of bias and inaccuracy by 10 years range of the samples of Bologna and Coimbra. In all tested methods the values of bias and inaccuracy increase after 49 years of age. Lower values are always found within this age limit.
However, in the whole sample there is a tendency to overestimate the age of individuals up to 50 years and underestimate over 50 years. Indeed, for mature and older adults there is a tendency to lower values of overestimation and to increased values of underestimation, which proportionally increase with increasing age, a situation that can be seen clearly in the case of older adults.

	PUBIC SYMPHYSIS			AURICULAR SURFACE			4° RIB			SUTURE CLOSURE		
	N.	BIAS	INAC.	N.	BIAS	INAC.	N.	BIAS	INAC.	N.	BIAS	INAC.
	MALE_BOLOGNA											
18-29	20	1,66	4,1	25	7,82	7,9	10	0,7	3,2	27	16,18	16,2
31-39	16	3,65	6,2	19	1,13	4,6	5	-4,9	5,4	17	8,4	8,01
40-49	13	3,1	6,6	16	-1,4	6,8	3	4,2	8,5	17	2,79	4,51
50-59	1	-8,25	9,8	22	-9,4	10,6	5	-12	15,6	24	-7,38	8,04
60+	43	-19,2	19,1	41	-19,7	19,3	10	-12,6	12,6	49	-22,4	22,4
	FEMALE_BOLOGNA											
18-29	17	4,2	5,2	23	5,9	6,1	11	-1,4	4,4	19	13,2	13,2
30-39	18	3,1	5,3	21	-0,6	1,9	6	-3,1	9,6	16	4,3	6,1
40-49	9	-0,2	5,2	12	-1,3	4,6	3	-0,2	3,5	11	-3,2	4,9
50-59	9	-11,5	11,5	14	-4,5	5,8	7	-16,2	16,4	13	-6,5	7,1
60+	28	-20,3	20,3	40	-18,4	18,3	5	-33,3	33,3	38	-25,5	25,5
	MALE _COIMBRA											
20-29	16	3,4	4,2	21	7,5	7,8	3	0	0	21	13,5	13,5
30-39	19	1,1	3,7	24	0,3	2,6	5	6,3	8,7	25	8,3	9,1
40-49	20	-2,1	4,1	23	-1,9	5	7	-1,4	4,8	23	3,7	4,9
50-59	21	-9,2	11,1	22	-6,2	6,5	8	-0,6	3,4	24	-5,1	5,9
60+	26	-17,5	17,7	23	-17,2	17,2	10	-9,1	9,1	27	-19,3	19,3
	FEMALE_COIMBRA											
19-29	14	3,1	4	16	10,3	10,3	6	-3,1	3,1	18	12,6	12,6
30-39	18	3,5	5	19	2,7	4,5	4	-0,6	4,4	19	4,8	5,6
40-49	13	0,4	2,3	15	-2,1	6,2	6	4,1	12,1	15	-3,9	5
50-59	19	-3,4	5,8	21	-5,4	6,8	2	4,3	8,8	21	-9,5	9,5
60+	45	-19,4	19,4	46	-20	20	15	-20,4	19	49	-27,2	27,2

Table 3.6. Index of bias and inaccuracy of tested methods by 10 years of the sample of Coimbra and Bologna separated by sex.

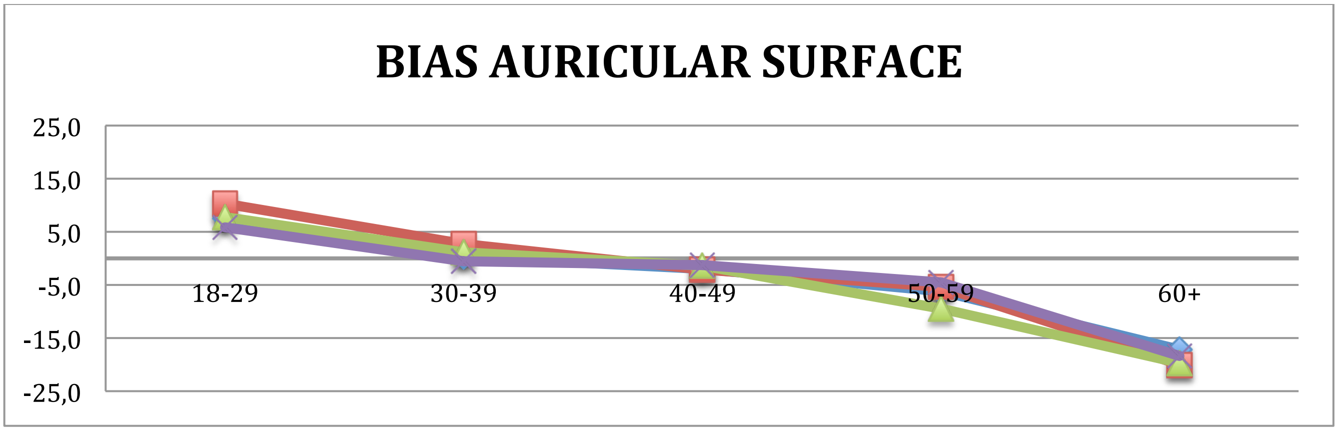
In the following graphs is represented the trend of the values of bias of the differents methods in the four subsample (MB green, FB purple, MC blu, FC red).

The method of pubic symphysis shows values of bias closer to zero in the female samples in the range from 40 to 49 years of age while the male samples have slightly higher values in the younger range (graph 3.1).



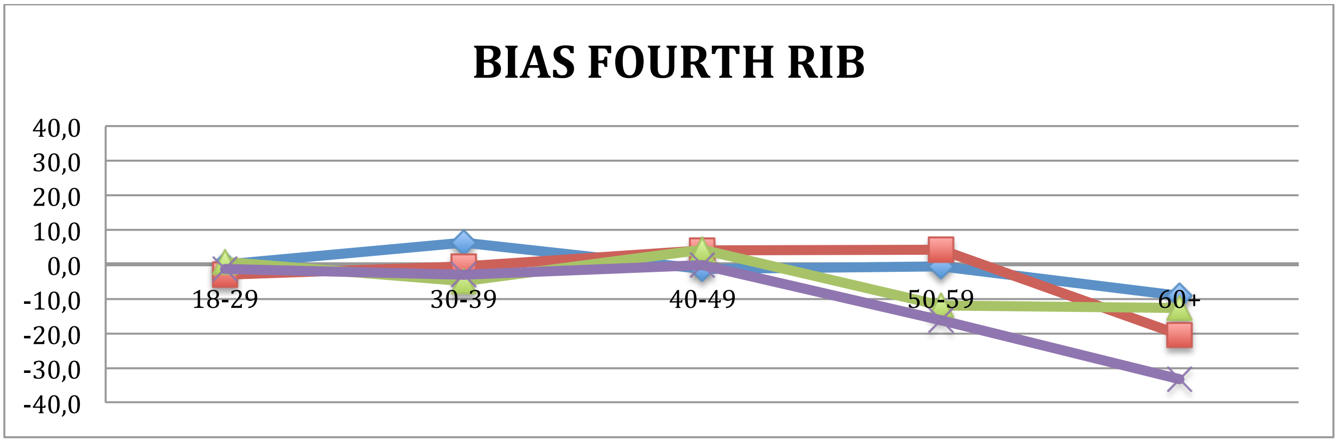
Graph 3.1 – trend of the values of bias in the sample to the pubic symphysis , values reported in table 3.6, on the axis of abscissae is reported the age range considered and on the axis of ordinates the bias values (MB green, FB purple, MC blu, FC red).

The method of the auricular surface shows values of bias closer to zero in the range from 30 to 39 in all samples except the females of Coimbra (graph 3.2).



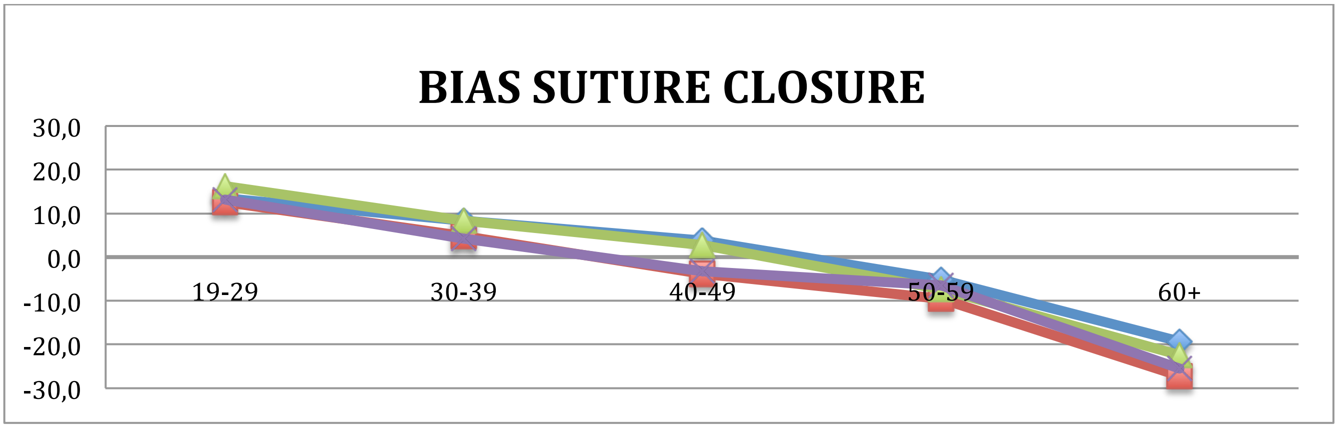
Graph 3.2 – trend of the values of bias in the sample for the method of the auricular surface, values reported in table 3.6, on the axis of abscissae is reported the age range considered and on the axis of ordinates the bias values (MB green, FB purple, MC blu, FC red).

The values of bias of the fourth rib show a more irregular trend than the other methods (graph 3.3). In the male sample of Coimbra we obtained a bias of zero in the range from 19 to 29, but it must be underlined that the data refers only to three individuals.



Graph 3.3 – trend of the values of bias in the sample for the method of the fourth rib, values reported in table 3.6, on the axis of abscissae is reported the age range considered and on the axis of ordinates the bias values (MB green, FB purple, MC blu, FC red).

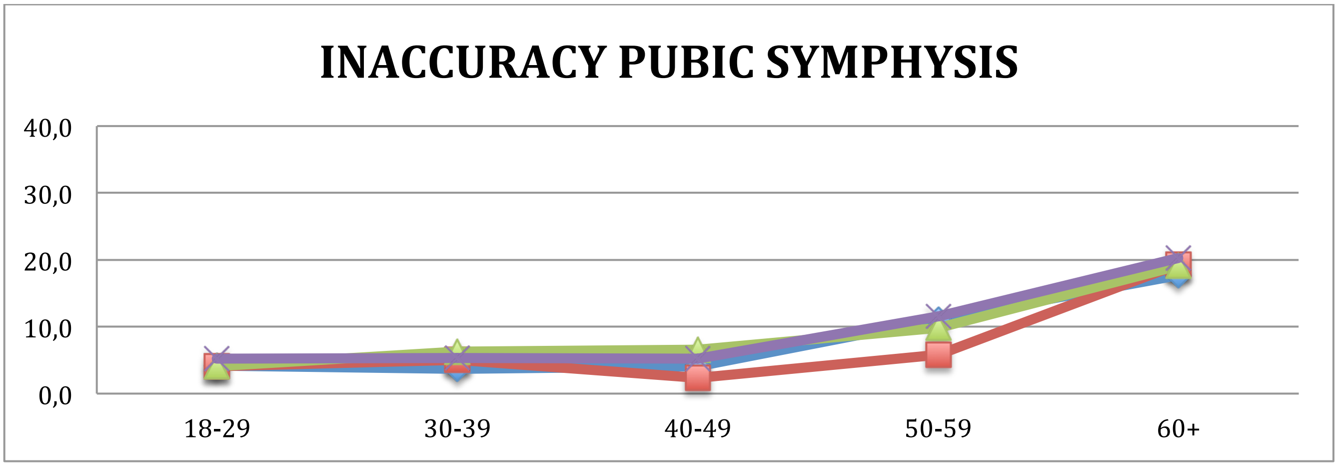
In the sutures closure method the values of bias show a similar trend in all samples, and are higher in comparison to the values of the pubic symphysis and the auricular surface. Value closer to zero are in the range 40-49 yars of age (graph 3.4).



Graph 3.4 – trend of the values of bias in the sample for the method of the suture closure, values reported in table 3.6, on the axis of abscissae is reported the age range considered and on the axis of ordinates the bias values (MB green, FB purple, MC blu, FC red).

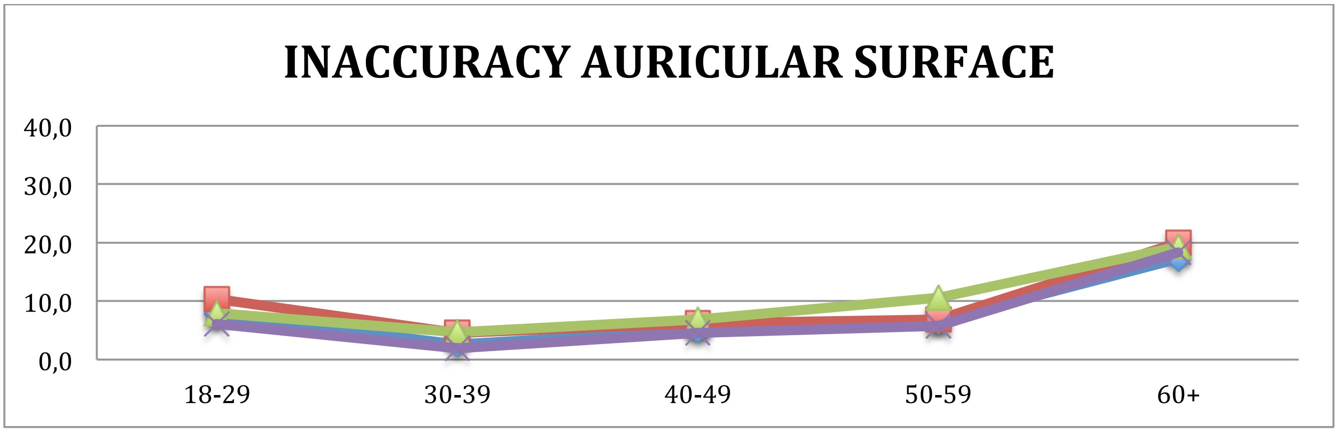
In the following graphs is represented the trend of the values of inaccuracy of the differents methods in the four subsample (MB, FB, MC, FC).

The values of inaccuracy of the pubic symphysis have a similar pattern in the samples and tend to increase after 50 years (graph 3.5).



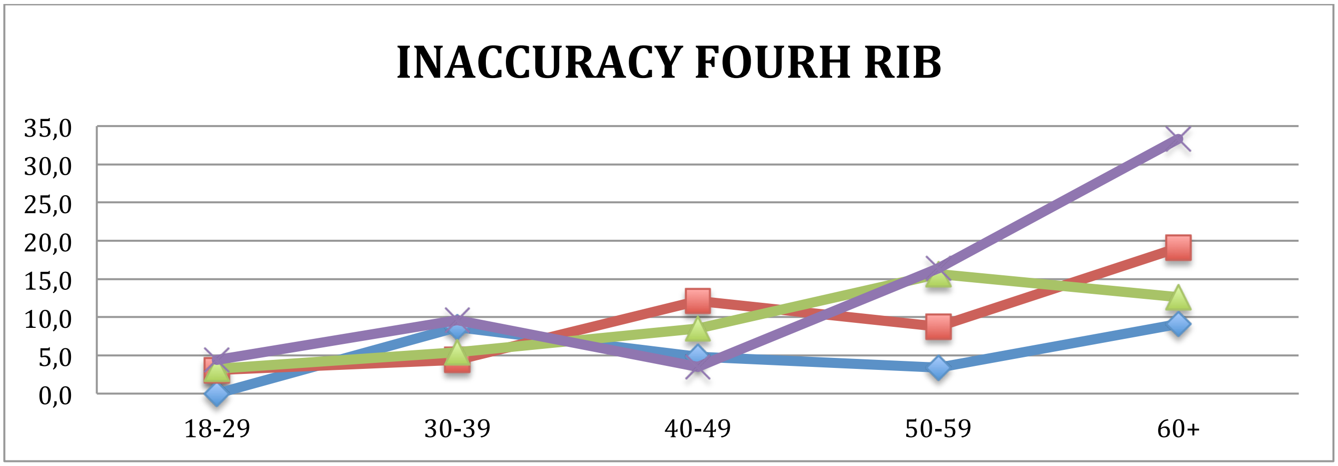
Graph 3.5– trend of the values of inaccuracy in the sample to the pubic symphysis, values reported in table 3.6, on the axis of abscissae is reported the age range considered and on the axis of ordinates the inaccuracy values (MB green, FB purple, MC blu, FC red).

The values of inaccuracy of the auricular surface have a trend almost overlapped and increase from 60 years in all samples (graph 3.6).



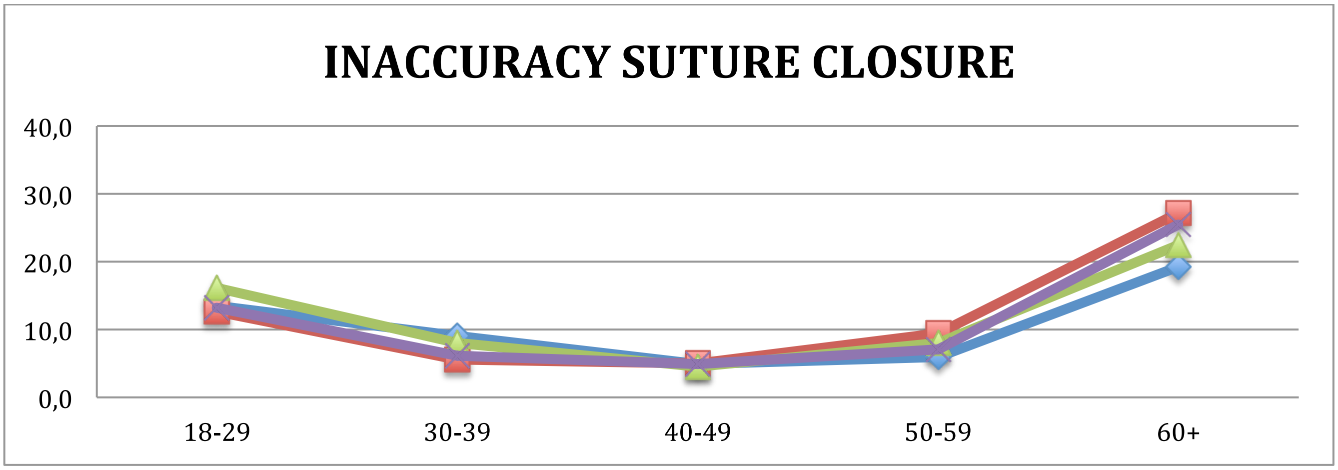
Graph 3.6 – trend of the values of inaccuracy in the sample for the method of the auricular surface, values reported in table 5, on the axis of abscissae is reported the age range considered and on the axis of ordinates the inaccuracy values (MB green, FB purple, MC blu, FC red).

The values of inaccuracy of the 4th rib show a trend more irregular than the other methods but they tend to exceed 10 years after 60 years (graph 3.7).



Graph 3.7 – trend of the values of inaccuracy in the sample for the method of the fourth rib, values reported in table 3.6, on the axis of abscissae is reported the age range considered and on the axis of ordinates the inaccuracy values (MB green, FB purple, MC blu, FC red).

In the sutures method the values of inaccuracy show a similar trend in all samples, the values are close to zero in the range 40-49 where we find an overestimation lower of 5 years. After 50 years the overestimation starts to increase and after 60 become more than 25 years (graph 3.8).



Graph 3.8 – trend of the values of inaccuracy in the sample for the method of the suture closure, values reported in table 3.6, on the axis of abscissae is reported the age range considered and on the axis of ordinates the inaccuracy values (MB green, FB purple, MC blu, FC red).

In the following table are represented the values of bias and inaccuracy of the lateral-anterior system, the vault and the average for the method of cranial suture closure.

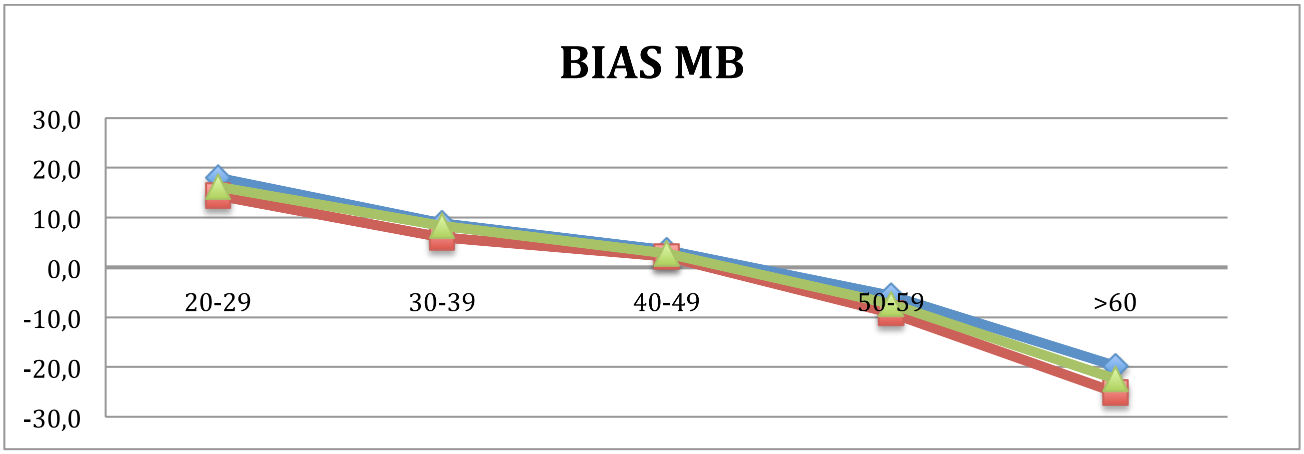
In the table 3.7 and graphs 3.9-3.16 we compare the results obtained in the study of the cranial sutures in the lateral-anterior system (blue), the vault (red) and the average (green) between the collections of Bologna and Coimbra for each sample.

In both subsample, male and female, the range 40-49 years show values closer to zero. The values of bias associated to each system tend to overlap in the sample of Bologna, especially in the male sample (graph 3.9 and 3.10). In the male sample of Coimbra instead there wasn't an overlap of the value of bias, while the female of Coimbra show a tendency similar to the female of Bologna (graph 3.11 and 3.12). In both samples the known age was underestimated over 50 years old. In the female samples overlap is limited to the 40-49 age range and the underestimation starts after 40 years old.

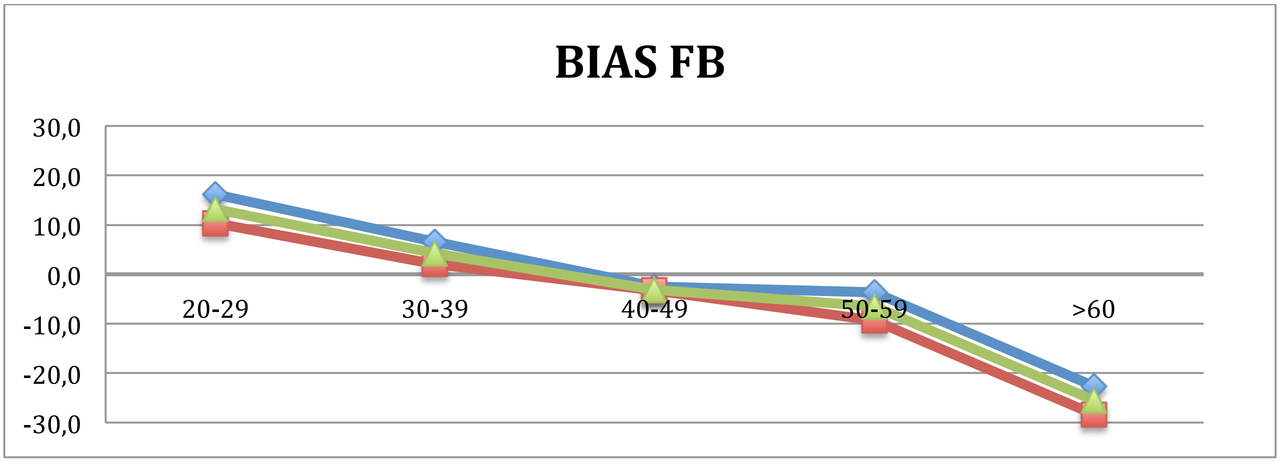
	BOLOGNA				COIMBRA			
	MALE		FEMALE		MALE		FEMALE	
	LATERAL-ANTERIOR SYSTEM							
	bias	inaccuracy	Bias	inaccuracy	bias	inaccuracy	bias	inaccuracy
20-29	18,0	18,0	16,1	16,1	16,4	16,4	14,5	12,6
30-39	8,8	8,8	6,5	7,8	10,4	11,2	8,0	5,6
40-49	3,5	5,3	-2,5	6,5	6,4	7,1	-2,3	5,0
50-59	-5,6	7,0	-3,6	4,4	-2,0	5,2	-7,3	9,5
>60	-19,8	19,8	-22,6	22,6	-15,7	15,7	-24,6	27,2
	VAULT							
20-29	14,4	14,4	10,3	10,3	10,6	10,6	10,8	10,2
30-39	6,0	6,4	2,1	5,2	6,2	7,0	1,9	4,5
40-49	2,1	3,7	-3,3	4,0	1,1	4,4	-5,5	6,4
50-59	-9,1	9,3	-9,4	9,4	-8,2	8,2	-11,7	12,6
>60	-25,1	25,1	-28,3	28,3	-22,8	22,8	-29,8	29,9
	AVERAGE: LATERAL-ANTERIOR SYSTEM-VAULT							
20-29	16,18	16,18	13,2	13,20	13,5	13,5	12,6	12,6
30-39	8,4	8,01	4,3	6,10	8,3	9,1	4,8	5,6
40-49	2,79	4,51	-3,2	4,90	3,7	4,9	-3,9	5,0
50-59	-7,38	8,04	-6,5	7,10	-5,1	5,9	-9,5	9,5
>60	-22,4	22,44	-25,5	25,50	-19,3	19,3	-27,2	27,2

Table 3.7 values of bias and inaccuracy of the lateral-anterior system, the vault and the average in the samples studied

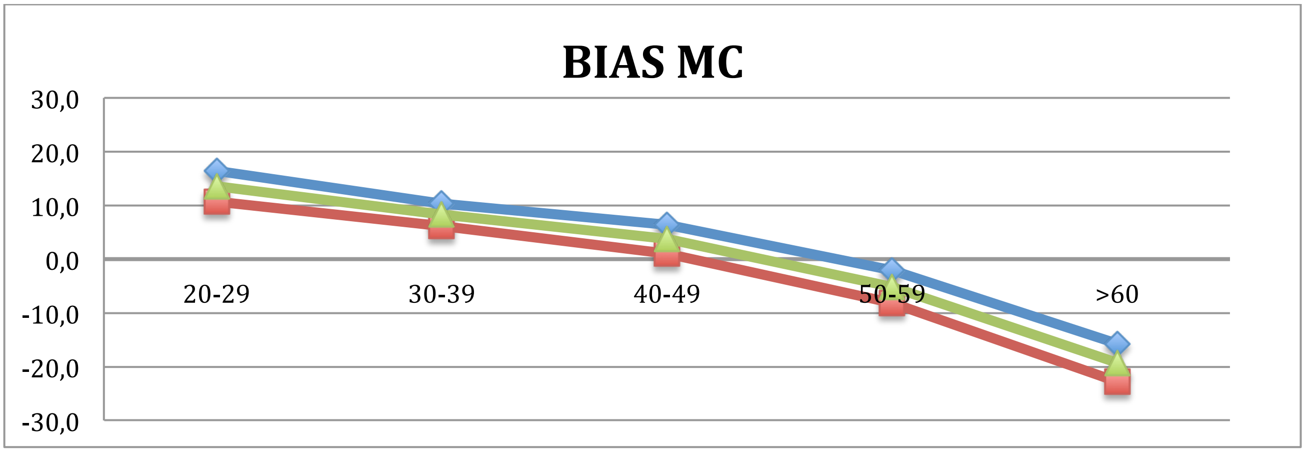
In the following graphs was represented the trend of the values of bias of the lateral-anterior system (blue), the vault (red) and the average (green) in the four subsample (MB, FB, MC, FC).



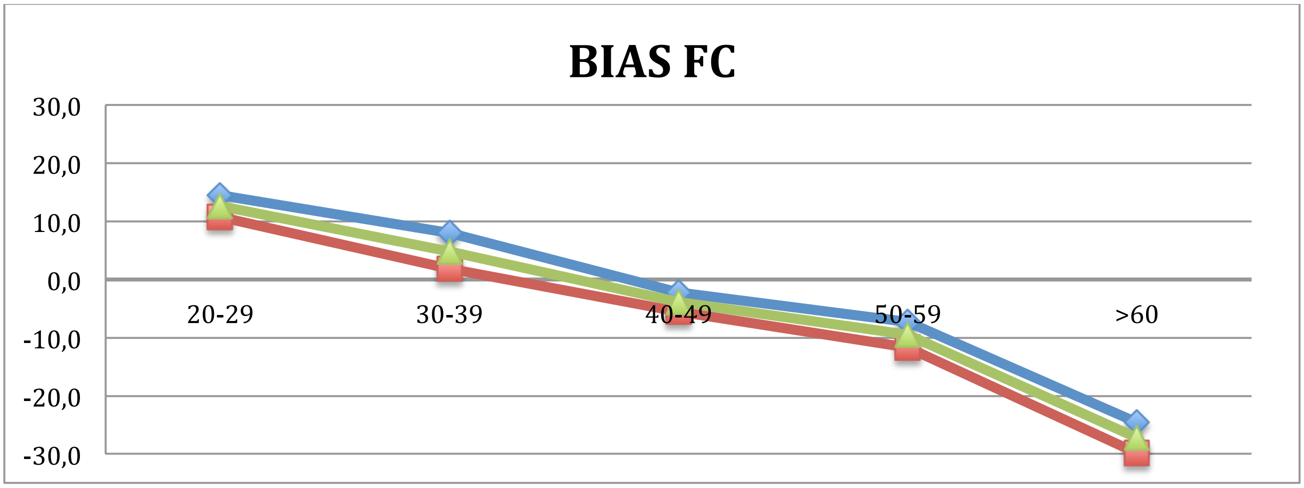
Graph 3.9 - trend of the values of bias of the lateral-anterior system, the vault and the average in the male sample of Bologna's collection for the method of the suture closure, on the axis of abscissae was reported the age range considered and on the axis of ordinates the bias values.



Graph 3.10- trend of the values of bias of the lateral-anterior system, the vault and the average in the female sample of Bologna's collection for the method of the suture closure, on the axis of abscissae was reported the age range considered and on the axis of ordinates the bias values.



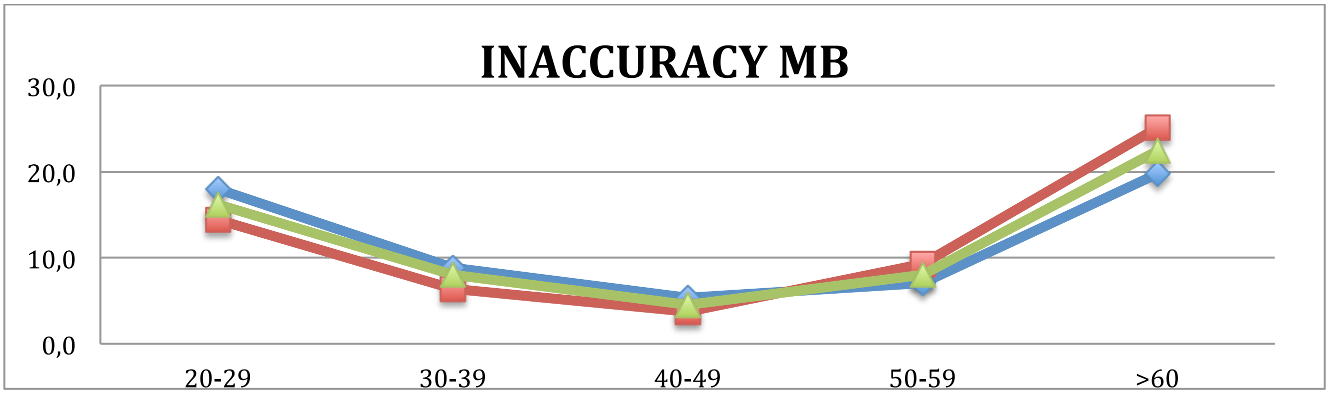
Graph 3.11- trend of the values of bias of the lateral-anterior system, the vault and the average in the male sample of Coimbra's collection for the method of the suture closure, on the axis of abscissae was reported the age range considered and on the axis of ordinates the bias values.



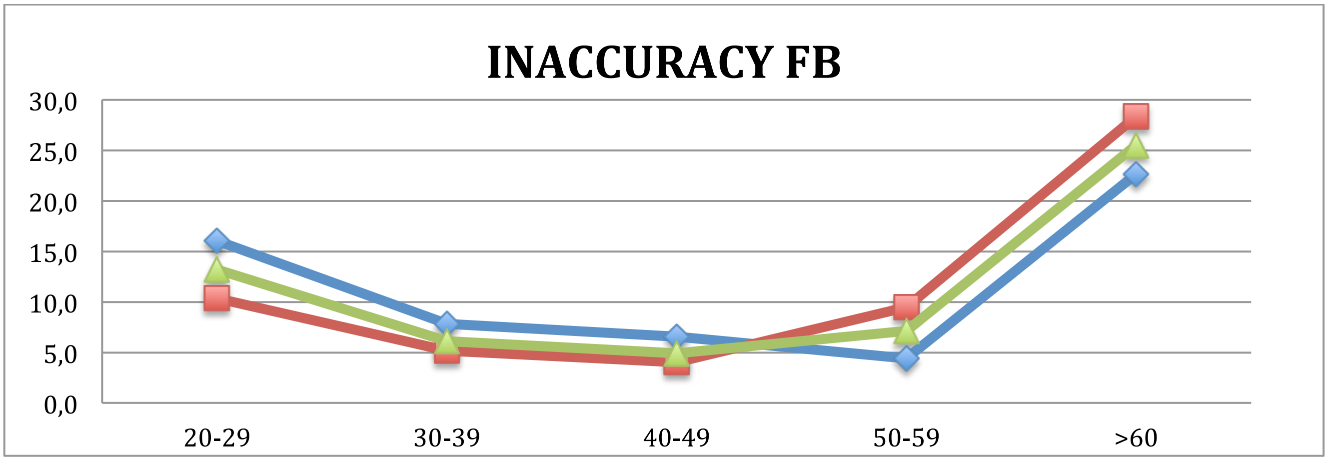
Graph 3.12- trend of the values of bias of the lateral-anterior system, the vault and the average in the female sample ofCoimbra's collection for the method of the suture closure, on the axis of abscissae was reported the age range considered and on the axis of ordinates the bias values.

In the following graphs was represented the trend of the values of inaccuracy of the lateral-anterior system (blue), the vault (red) and the average (green) in the four subsample (MB, FB, MC, FC).

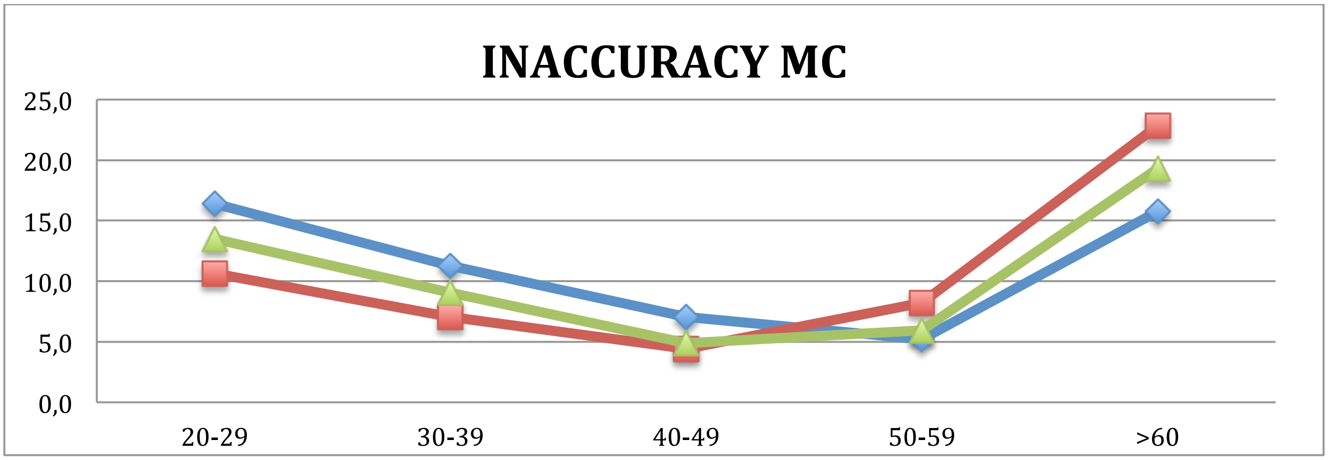
For what concern the values of inaccuracy the range with the lowest values was between 40-49 years in the sample of Bologna and 40-60 years in the sample of Coimbra; in the female sample the range with the lowest values was between 30-49 years.



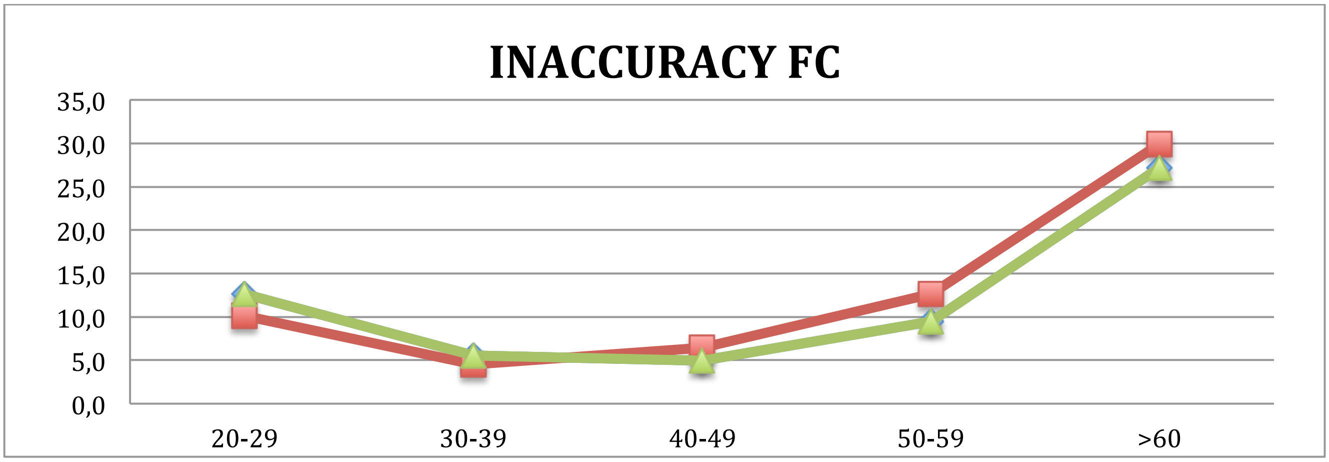
Graph 3.13 trend of the values of inaccuracy of the lateral-anterior system, the vault and the average in the male sample of Bologna's collection for the method of the suture closure, on the axis of abscissae was reported the age range considered and on the axis of ordinates the inaccuracy values.



Graph 3.14 trend of the values of inaccuracy of the lateral-anterior system, the vault and the average in the female sample of Bologna's collection for the method of the suture closure, on the axis of abscissae was reported the age range considered and on the axis of ordinates the inaccuracy values.



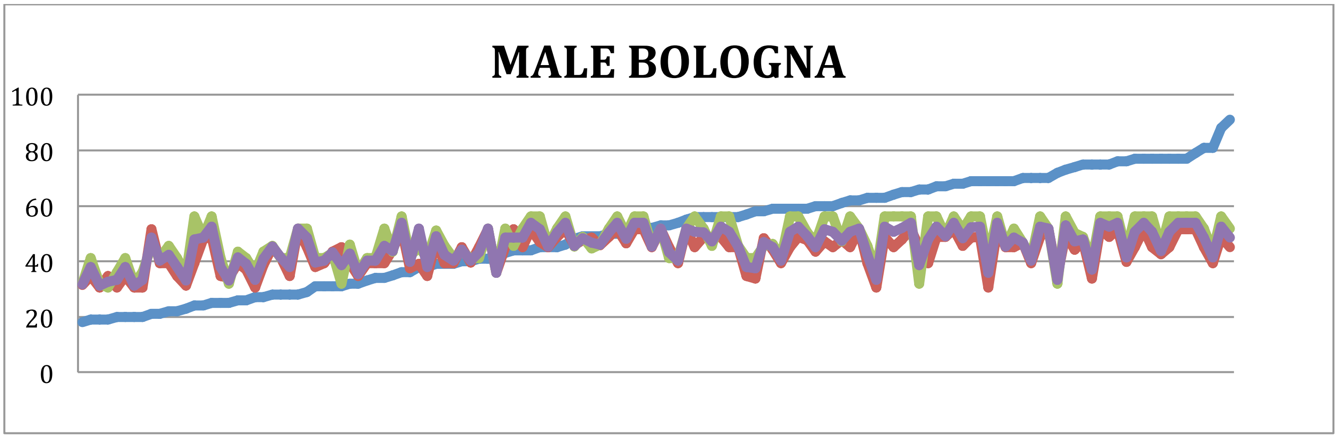
Graph 3.15 trend of the values of inaccuracy of the lateral-anterior system, the vault and the average in the male sample of Coimbra's collection for the method of the suture closure, on the axis of abscissae was reported the age range considered and on the axis of ordinates the inaccuracy values.



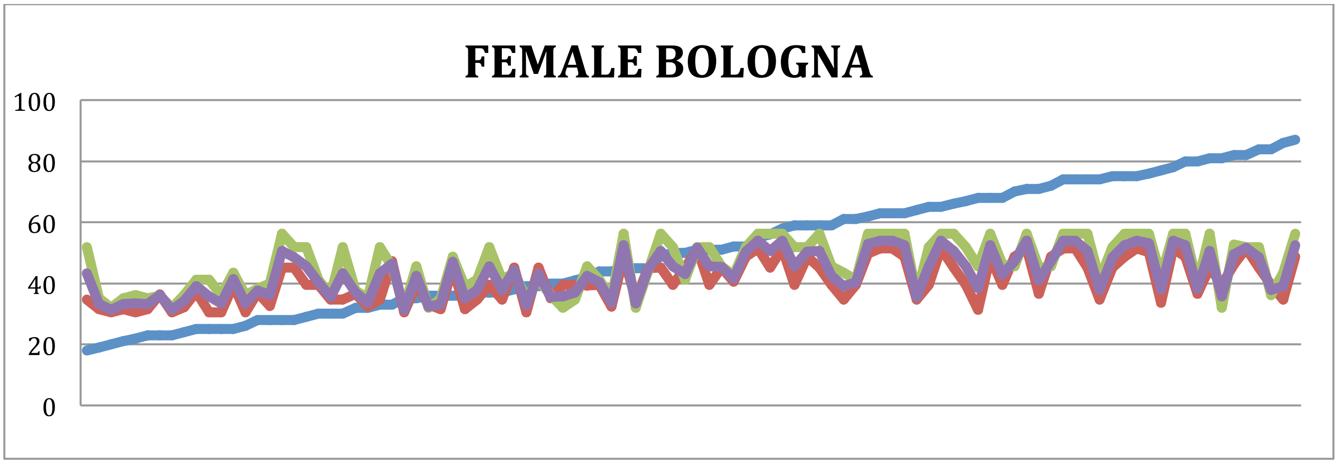
Graph 3.16 trend of the values of inaccuracy of the lateral-anterior system, the vault and the average in the female sample of Coimbra's collection for the method of the suture closure, on the axis of abscissae was reported the age range considered and on the axis of ordinates the inaccuracy values.

The values of bias and inaccuracy of the lateral-anterior system, the vault and the average were lower in the female samples in both collection, especially in the 20-39 years age range. In the following graphs (3.17 , 3.18, 3.19, 3.20) the estimated age from the anterior-lateral system, the vault system and the average of the two system of each individual are reported in detail, compared to known age (individuals ordered from the youngest to the oldest).

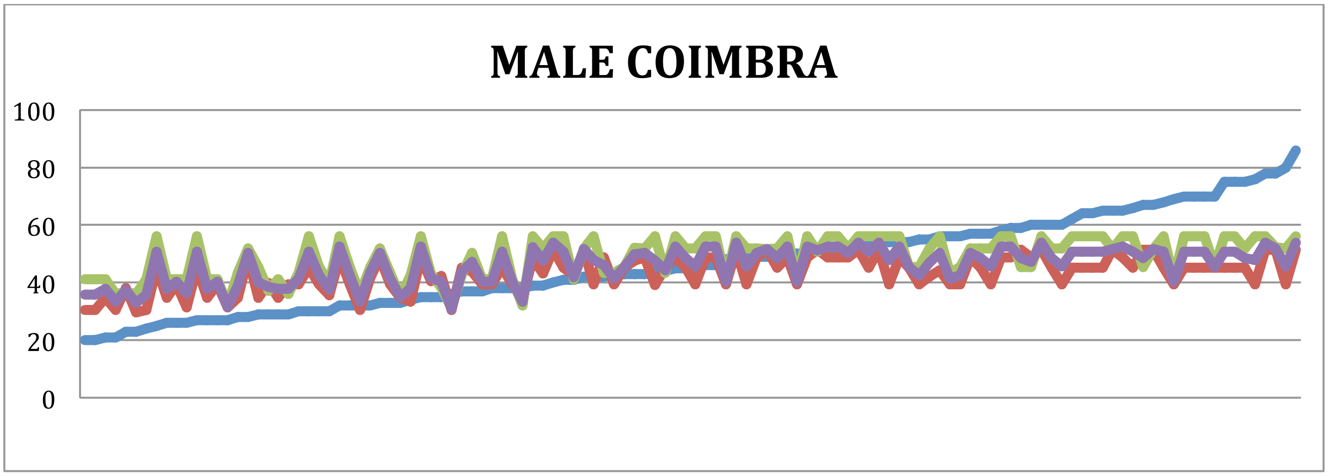
The anterior-lateral system consistently gives higher age estimates compared to the vault system, although the system of the vault, the average and the lateral-anterior system tend to overlap without big differences. The method appears to be more reliable in the range 40-50 years in the whole sample with the exception of the males of Coimbra were the higher reliability was in the range 50-60 years.



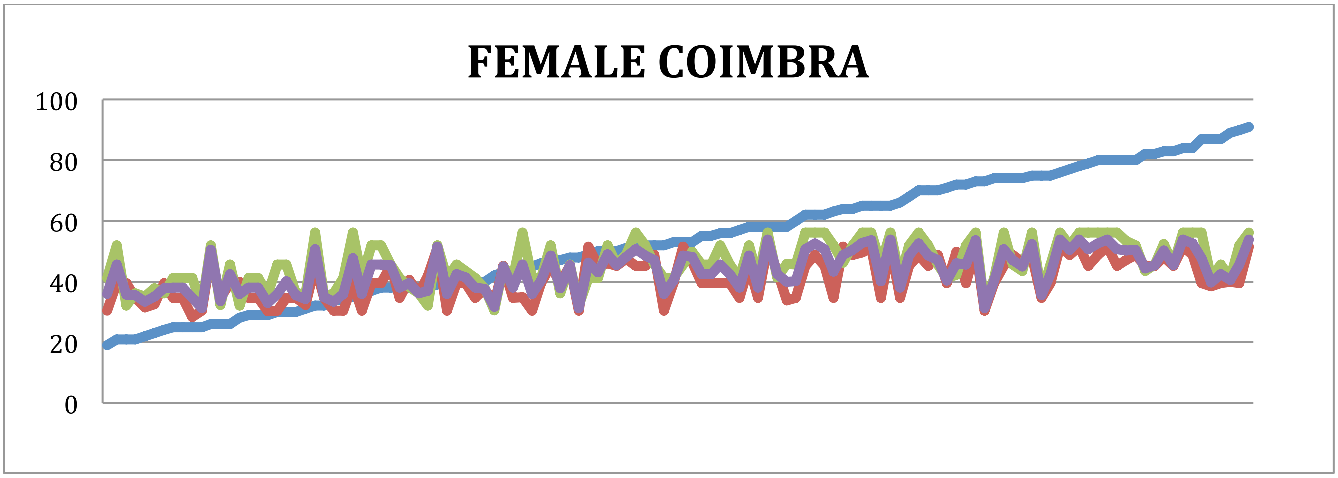
Graph 3.17- comparison beetween the known age (blue) and the estimated age from the lateral-anterior system (green), the vault (red) and the average (purple) for each male of Bologna.The individuals are on the axis of abscissae (ID number not shown because they are too much), the age is on the axis of ordinates.



Graph 3.18 comparison beetween the known age (blue) and the estimated age from the lateral-anterior system (green), the vault (red) and the average (purple) for each female of Bologna. The individuals are on the axis of abscissae (ID number not shown because they are too much), the age is on the axis of ordinates.



Graph 3.19 comparison beetween the known age (blue) and the estimated age from the lateral-anterior system (green), the vault (red) and the average (purple) for each male of Coimbra. The individuals are on the axis of abscissae (ID number not shown because they are too much), the age is on the axis of ordinates.



Graph 3.20. comparison beetween the known age (blue) and the estimated age from the lateral-anterior system (green), the vault (red) and the average (purple) for each female of Coimbra. The individuals are on the axis of abscissae (ID number not shown because they are too much), the age is on the axis of ordinates.

- study of laterality

For each individual, the right and the left side of the pelvis were studied separately and then compared (table 3.8) while laterality of the fourth rib was not considered because of the low sample size.
Laterality for sutures was not considered because this method for takes into account only one of equal points (the method does not specify wich side consider so it was decided to take the one with the highest value of synostosis).

	PUBIC SYMPHYSIS		AURICULAR SURFACE	
	N.	%	N.	%
MALE BOLOGNA				
R=L	57	75	69	64
R≠L	19	25	38	36
TOT M	76		107	
FEMALE BOLOGNA				
R=L	39	74	72	71
R≠L	14	26	29	29
TOT F	53		101	
TOT B	129		208	
MALE COIMBRA				
R=L	81	93	77	75
R≠L	6	7	25	25
TOT M	87		102	
FEMALE COIMBRA				
R=L	79	81	82	75
R≠L	19	19	27	25
TOT F	98		109	
TOT C	185		211	
TOT B+C	314		419	

Table 3.8 comparation of the results of the pubic symphysis and auricular surface on the right and left side of the pelvis.

The same degree was assigned on both sides of the pubic symphysis in 74% of individuals of Bologna and 86% of the sample of Coimbra and respectively in 68% and 75 % for to the auricular surface.
The sample of Coimbra shows percentages of concordance between the two sides higher than Bologna.

- variation of the degree of sacral vertebral fusion (Belcastro et al. 2008)

Here are reported the results of the fusion of the ventral face of sacral vertebral bodies in the individual of the collection of Bologna. In this study were not considered the incomplete and patological sacra. The sample is describe in table 3.9. The method was not applied on the Coimbra collection because this method was developed on this collection.

	N.	age range
M	86	18-91
F	73	18-86

Table 3.9 sample examinated

The absence of fusion (degree 0) is present in the site S1–2 in both sample for YA, and only in one individual in the male sample of MA and only in one female in OA; in the site S2-3 is present in 2 individuals. In the male sample degree 0 is most found in the site S2–3 and S3–4 with 100% of frequency in MA and OA.

Degrees of incomplete fusion (degrees 1, 2) are found in the site S1–2 in both samples for YA. For sites S2–3, S3–4 and S4–5 degree 1 is present in 1-2 individuals only in YA. Degree 2 is found in YA except for one individual (MA, male) and in all age class in the female sample. In the female sample is present a higher % of degree 2 in all age range for all sites except S2-3; in the male sample this % is consistently lower. Degree 3 (complete fusion) is present in all sites in all age class and is the most frequent in males and females for site S2–3 and in males for S3–4; for the site S1–2, degree 3 is the most frequent starting from 35 years of age. The complete fusion increase with ageing in all sites in both samples. Site S1– 2 shows the highest variability of fusion degrees in all subsamples. This variability is particularly evident in individuals younger than 35 years.

		Bologna							
		MALE				FEMALE			
		0	1	2	3	0	1	2	3
S1-S2	YA	21% (6/29)	21% (6/29)	31% (9/29)	28% (8/29)	9% (2/23)	30% (7/23)	30% (7/23)	30% (7/23)
	MA	6% (1/16)	-	6% (1/16)	87% (14/16)	-	6%(1/17)	24% (4/17)	71% (12/17)
	OA	-	5% (2/41)	2% (2/41)	93% (38/41)	3% (1/33)	9%(3/33)	15% (5/33)	73% (24/33)
S2-S3	YA	7% (2/29)	-	17%(5/29)	76% (19/23)	-	-	17% (4/23)	83% (19/23)
	MA	-	-	-	100% (16/16)	-	-	-	100% (17/17)
	OA	-	-	-	100% (41/41)	-	-	6% (2/33)	94% (31/33)
S3-S4	YA	-	1% (1/29)	7% (2/29)	90% (26/29)	-	4% (1/23)	22% (5/23)	74% (17/23)
	MA	-	-	-	100% (16/16)	-	-	18% (3/17)	82% (14/17)
	OA	-	-	-	100% (41/41)	-	-	9% (3/33)	91% (30/33)
S4-S5	YA	-	7% (2/29)	7% (2/29)	86% (25/29)	-	4% (1/23)	43% (10/23)	52% (12/23)
	MA	-	-	6% (1/16)	94% (15/16)	-	-	12% (2/17)	88% (15/17)
	OA	-	-	-	100% (41/41)	-	-	12% (4/33)	88% (29/33)

Table 3.10 percentage of different grade of fusions of each site of the sacra

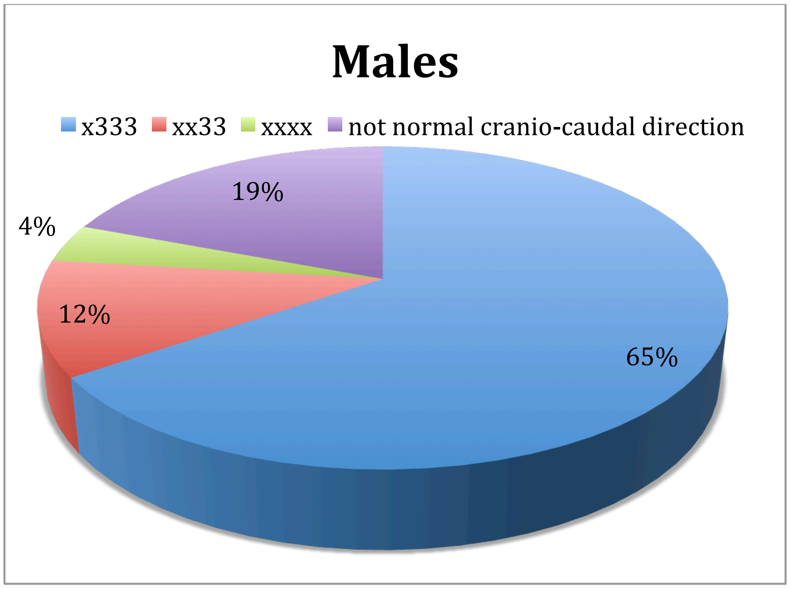
Sacra were classified according to the four groups identified by Belcastro et al (2008):
- (a) type ‘‘x333’’ (0333, 1333, 2333, 3333);
- (b) type “xx33” complete fusion only in the lower half (0133, 1233, . . .) or at the last site “xxx3” (1223, 1123, . . .);
- (c) type ‘‘xxxx’’ sacra in which none of the sites presents Degree 3 (without considering the order of fusion) (0111, 1222, 2121, ...);
- (d) patterns that do not show the normal caudo-cranial direction of sacral fusion but which contain at least one completely fused site (3332, 3010, . . .).

The relative abundances of each group are represented in table 3.11 and in graph 3.21-3.22.

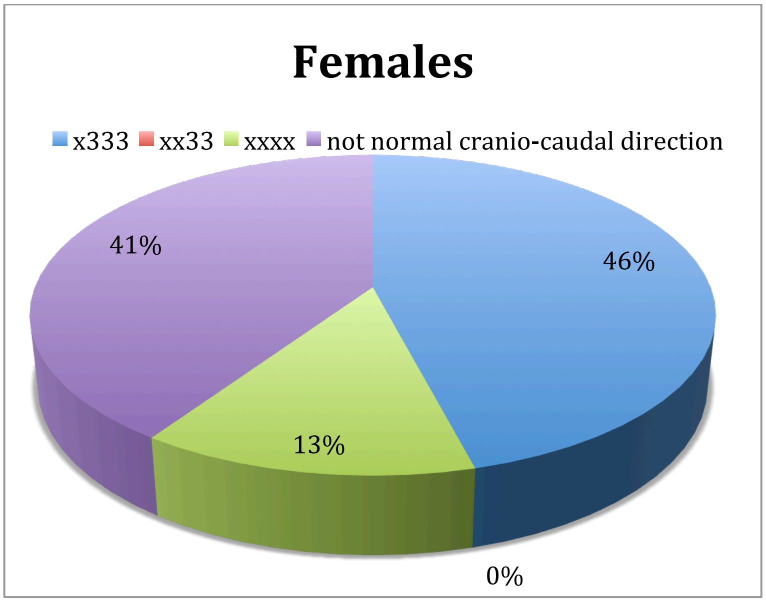
The a) pattern was the most frequent, in particulary in the male sample (90%); within this group the pattern “3333” is the most frequent with 70% (60/86) in the male sample and 49% (36/73) in the female sample. In the male sample the group a) is present only in 3/53 individuals after 38 years (2 individuals with 1333, one individual with 2333). In the female sample the pattern is more variable compared to the male sample, in the d) group are present different combination of fusion stage.

	male	female
a) x333	90% (77/86)	73% (53/73)
b) xx33	3% (3/86)	0
c) xxxx	1% (1(86)	7% (5/73)
d) not normal cranio-caudal direction	6% (5/86)	21% (15/73)

Table 3.11 frequency of each groups in male and female of Bologna’s collection



Graph 3.21 percentance of each groups in male of Bologna’s collection



Graph 3.22 percentance of each groups in female of Bologna's collection

	Male			Female		
	YA	MA	OA	YA	MA	OA
upper score	2,18	2,89	2,94	2,33	2,82	2,76
lower score	2,84	2,96	3,00	2,59	2,85	2,89
total score	2,51	2,93	2,97	2,46	2,84	2,83

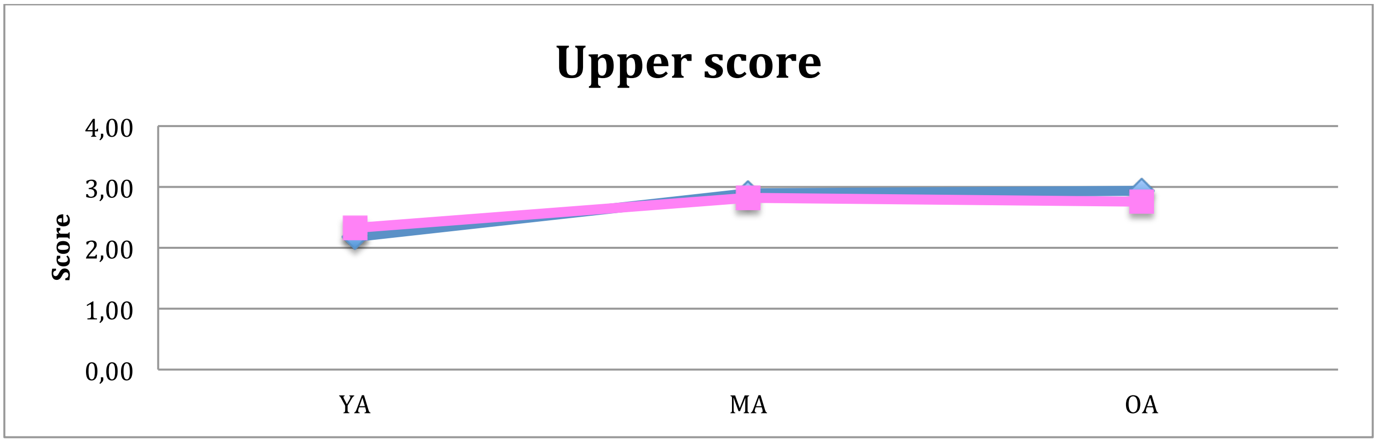
Table 3.12 upper score, lower score and total score of the male and female sample

The score tends to increase with age in both samples. The lower part of the sacrum presents less variability and the lower score is associated with the maximun value of score in both samples.

The male sample shows higher scores values than the female sample for the same age class.

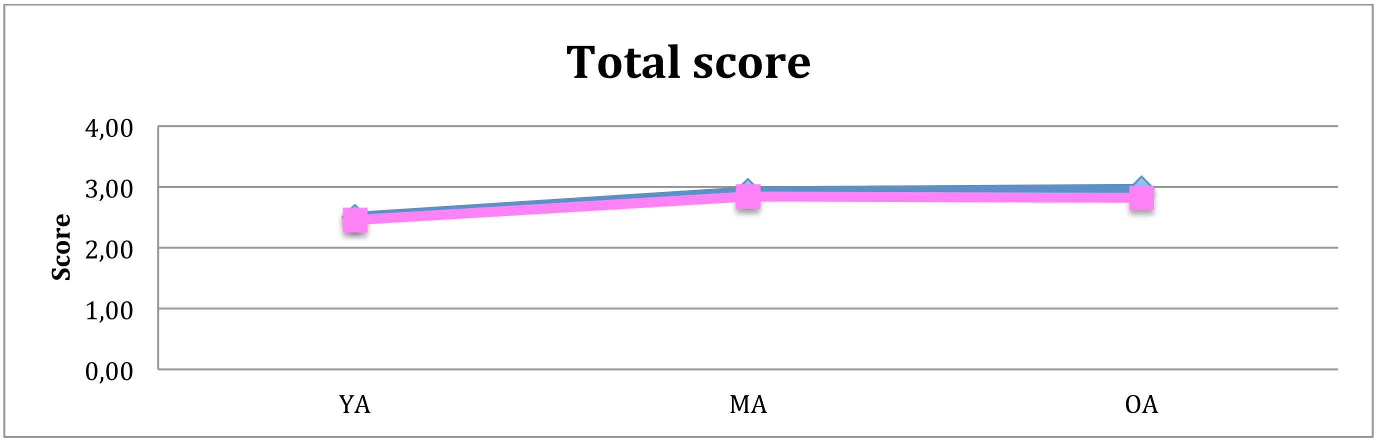
The minimum score value was found in YA males (2,18) while the maximun score value was found in OA males.

Graphs 3.23 - 3.24 – 3.25 illustrate the mean values of the upper, lower and total score of the males (blu) and females (pink) divided according to age classes. The curves of the scores of male and female were similar although the lower part of the sacrum presents higher scores values.



Graph 3.23 Mean values of the upper scores of SVF for YA, MA, OA age classes of males and females

Graph 3.24. Mean values of the lower scores of SVF for YA, MA, OA age classes of males and females



Graph 3.25 Mean values of the total scores of SVF for YA, MA, OA age classes of males and females

3.2) Sex determination

The samples of Bologna and Coimbra are balanced in sex ratio (Pχ2= 0,261) (table 3.13).

COLLECTION	M+F	M	F
BOLOGNA	247	135	112
COIMBRA	242	120	122
TOT.	489	255	234

Table 3.13. sample examinated

For 488 individuals it was possible to calculated the index of sexualization (Acsadi e Nemerkeri, 1970) of the skull and pelvis and the average of the two values; the Phenice method was also applied to the pelvis of 418 individuals. The remain of the sample was not included due to poor preservation of the interested districts.

- coefficient of sexualization (Acsadi e Nemerkeri, 1970)

The index of sexualization obtained by considering the skull and pelvis together allows a correct diagnosis in all samples, except for two females of Coimbra (table 3.14). The sex is confirmed in more than 99% of the total sample (486/488) (fig. 3.2.1).

In 469 individuals skull and pelvis report the same sex diagnosis and confim the known sex; in 19 individuals the two districts report a different diagnosis: in 84% of cases the skull is the district that gives a wrong result. However the global index of sexualization confirms the known sex in about 17 out of 19 individuals.

Figure 3.2.1 skull and left coxal bone of Bologna female n.4 (41 years old)

	FB			MB			FC			MC		
	MATCH		MISMATCH	MATCH		MISMATCH	MATCH		MISMATCH	MATCH		MISMATCH
	N.	%	N.	N.	%	N.	N.	%	N.	N.	%	N.
SKULL	103	96	4	130	96	5	118	97	4	117	97	3
PELVIS	110	100	-	128	99	1	120	98	2	120	100	-
SKULL+ PELVIS	111	100	-	135	100	-	120	98	2	120	100	-

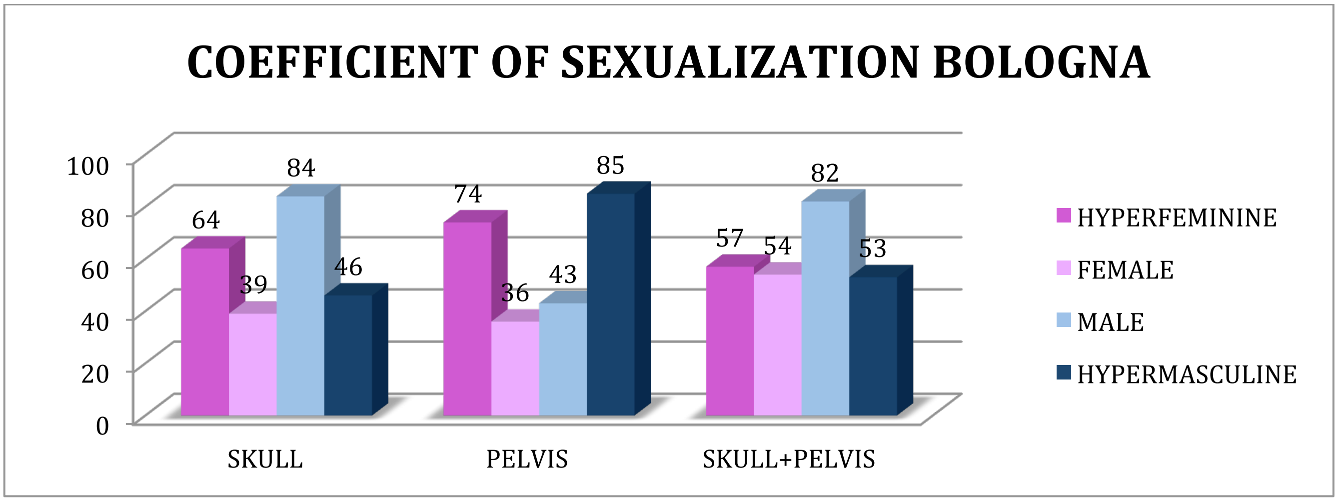
Table 3.14. number of matches and mismatches among known and attributed sex.

The table 3.15 shows the number of individuals in the two samples with values ranging from -2 to -1 (hyperfeminine), from -0.99 to 0 (female), from 0 to 0.99 (male), from 1 to 2 (hypermasculine). The individuals with only one district were included. The minimum and maximum value found in each range are also indicated.

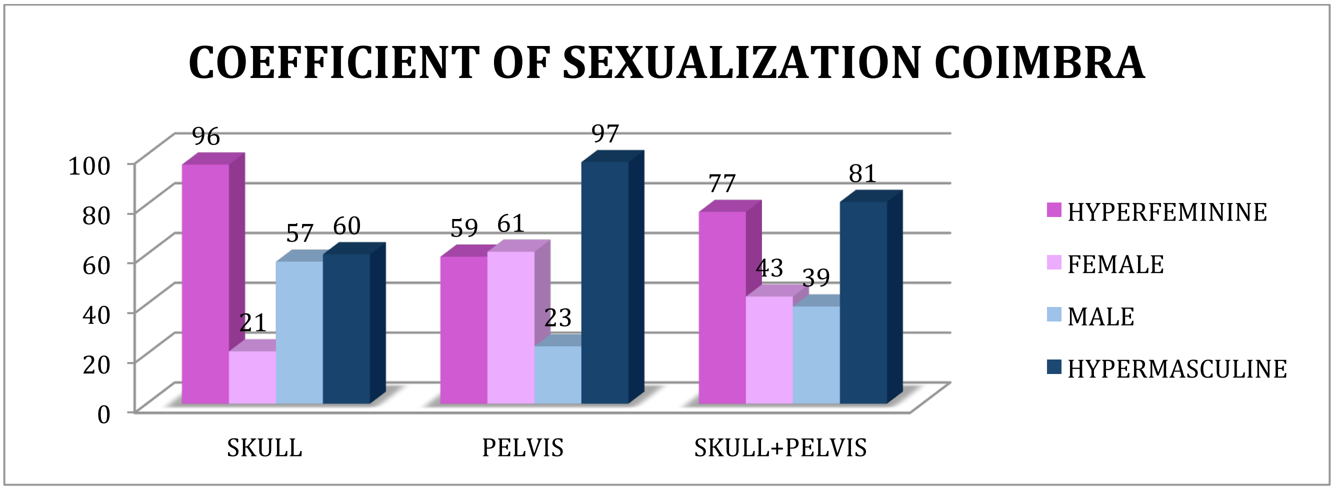
	BOLOGNA											
	FEMALE						MALE					
	-2_-1			-0,99_0			0_0,99			1_2		
	%	N.	MIN/MAX	%	N.	MIN/MAX	%	N.	MIN/MAX	%	N.	MIN/MAX
SKULL	62	64	-1_-1,82	38	39	-0,11_-0,89	65	84	0,03_0,97	35	46	1_1,68
PELVIS	67	74	-1_-2	33	36	-0,05_-0,95	34	43	0,05_0,95	66	85	1_1,8
SKULL+PELVIS	51	57	-1_-1,78	49	54	-0,17_-0,98	61	82	0,03_0,99	39	53	1_1,66
	COIMBRA											
SKULL	82	96	-1_-2	18	22	0_-0,96	45	57	0,25_0,96	55	60	1_1,68
PELVIS	49	59	-1_-1,89	51	61	0_-0,95	19	23	0_0,95	81	97	1_1,75
SKULL+PELVIS	64	77	-1_-1,68	36	43	-0,03_-0,95	32	39	0,19_0,98	68	81	1,03_1,72

Table 3.15 number of individuals in the two samples with values between -2 and -1 (hyperfeminine), -0.99 and 0 (female), 0 and 0.99 (male), 1 and 2 (hypermasculine); the extreme coefficients of each range are indicated in brackets.

Graphs 3.26 and 3.27 show the number of hyperfeminine, female, male, hypermasculine individuals in the sample of Bologna (3.26) and Coimbra (3.27).



Graph 3.26 Number of hyperfeminine, female, male, hypermasculine individuals in the sample of Bologna.



Graph 3.27 number of hyperfeminine, female, male, hypermasculine individuals in the sample of Coimbra.

From the graphs we can observe that the pelvis shows the more distinct sexual dimorphism, especially the masculine characters, particularly in the sample of Coimbra.

From the comparison of the two graphs it can be noticed that the population of Coimbra presents more marked sex characters both in the male and female sample: 96/122 female presents a hyperfeminine skull and 60/120 male presents a hypermasculine skull.

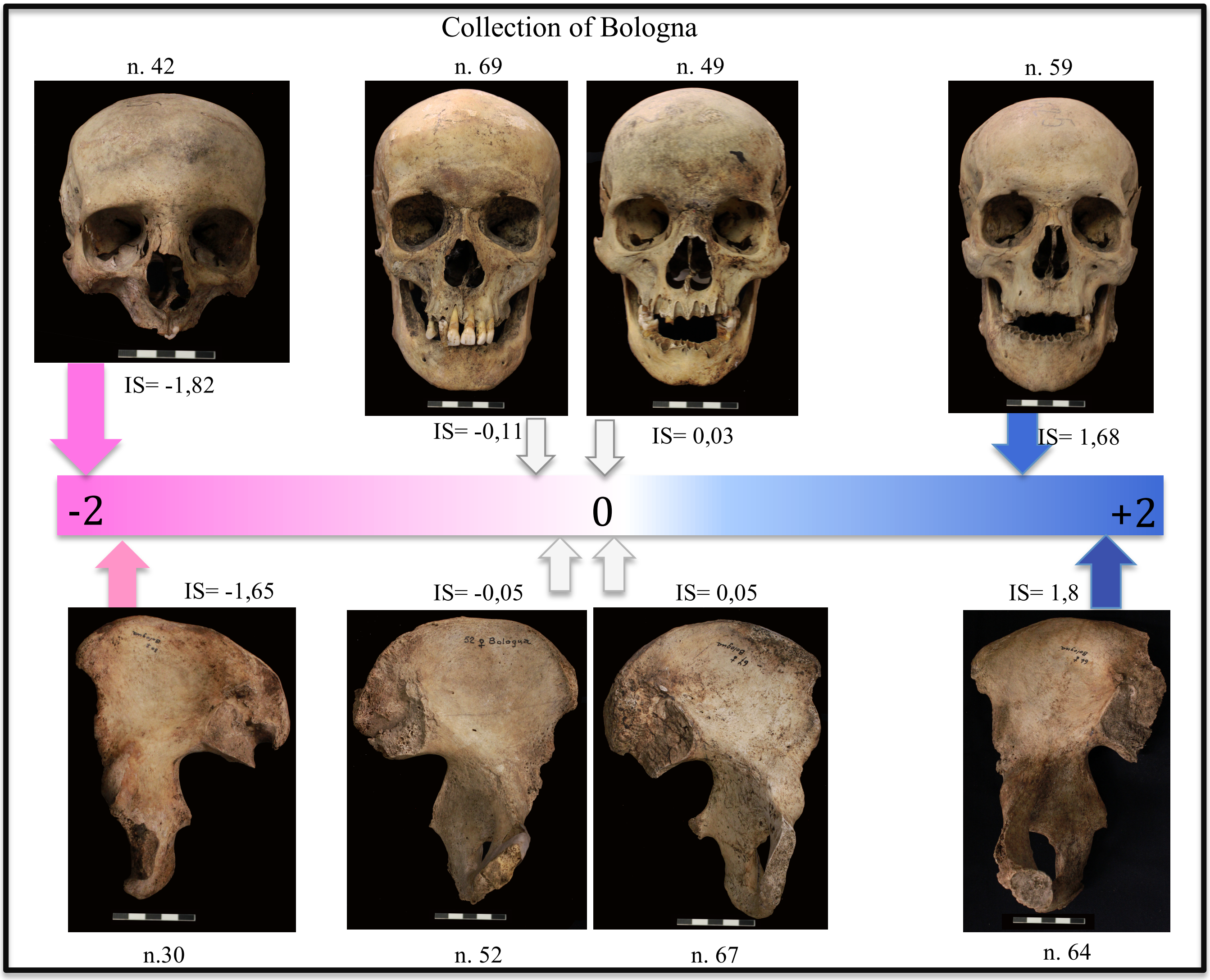


Figure 3.2.2 Sexual dimorphism in the collection of Bologna the values ranging from -2 to -1 (hyperfeminine), from -0.99 to 0 (female), from 0 to 0.99 (male), from 1 to 2 (hypermasculine), a IS score equals or approaching to zero, must be regarded as uncertain sex (Ascadi and Nemeskeri, 1970).

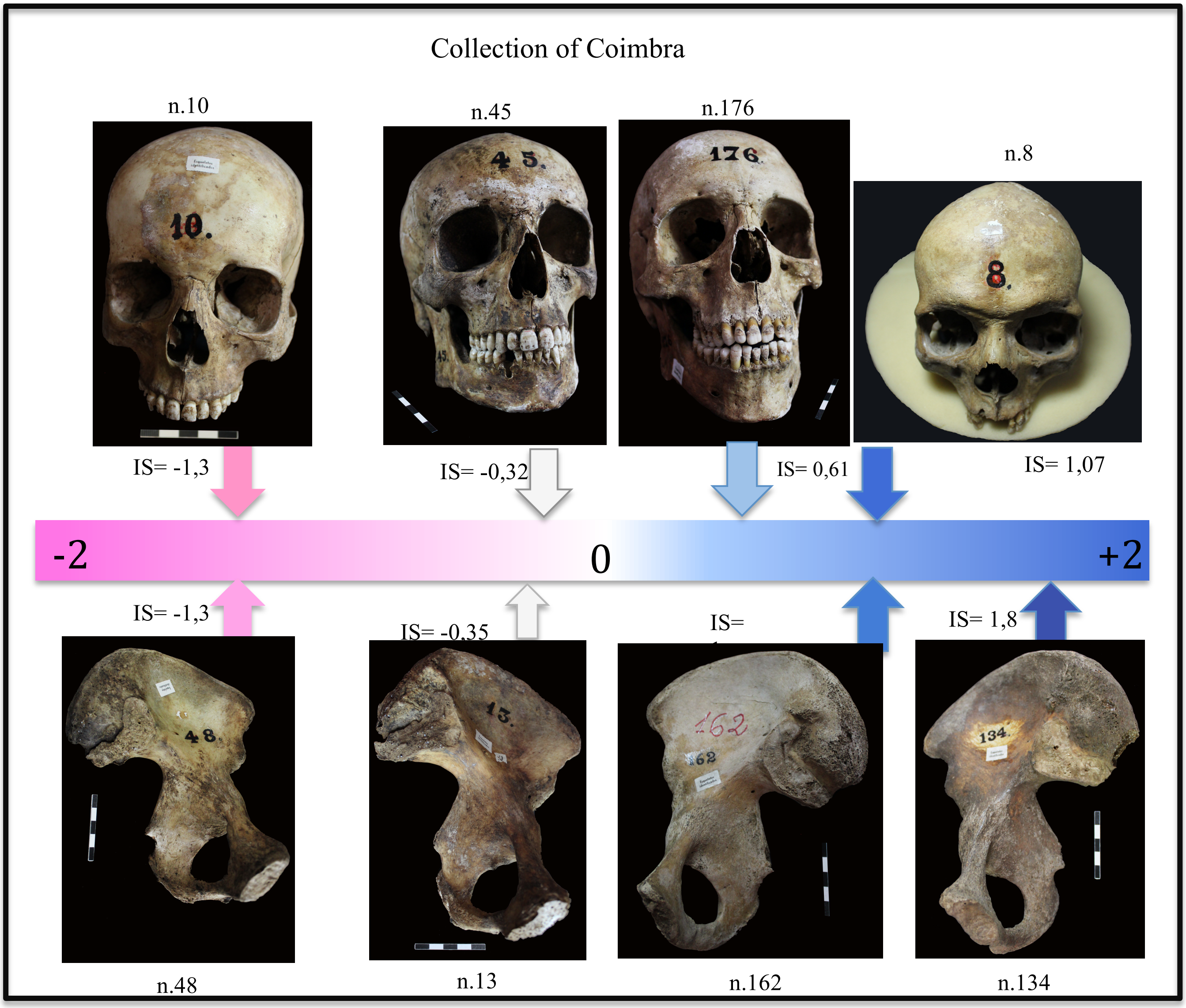
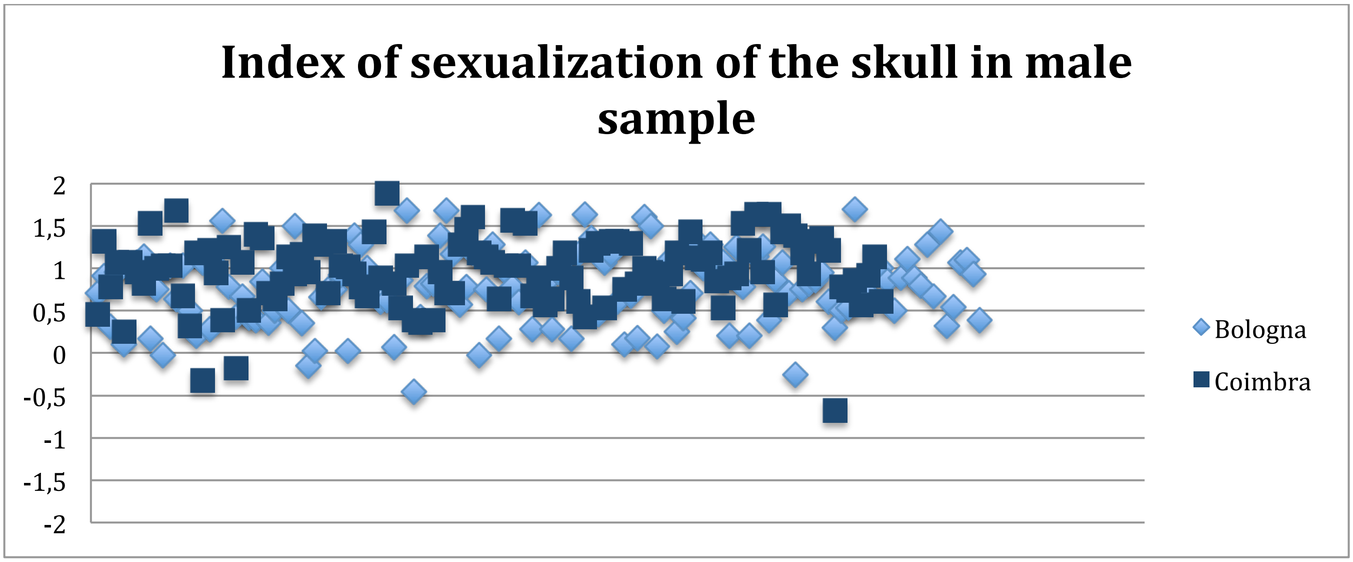
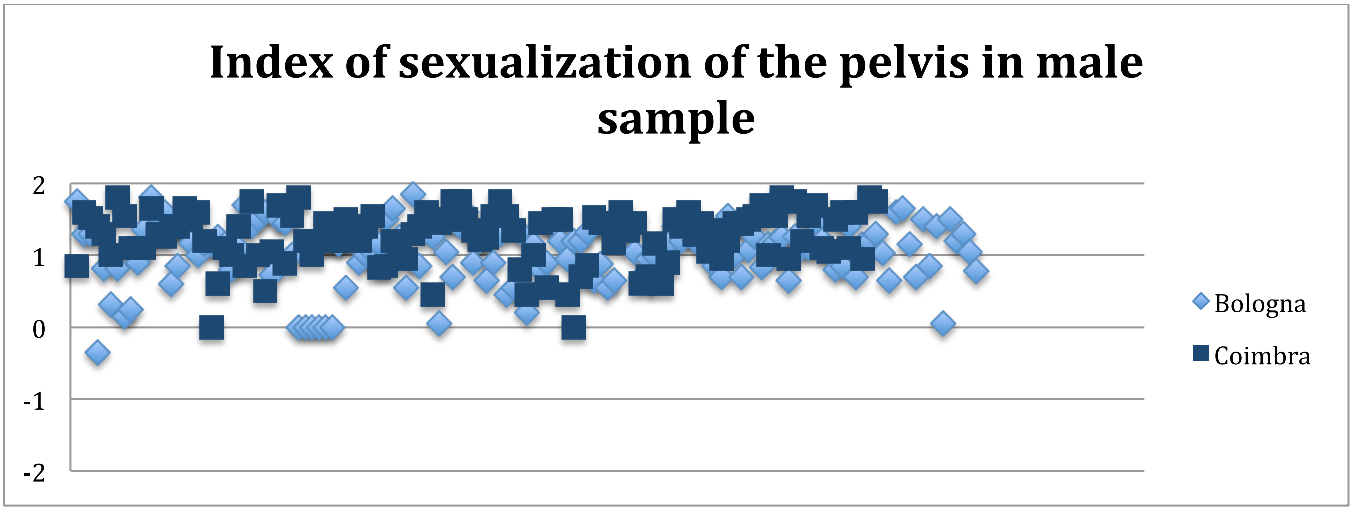


Figure 3.2.3 Sexual dimorphism in the collection of Coimbra the values ranging from -2 to -1 (hyperfeminine), from -0.99 to 0 (female), from 0 to 0.99 (male), from 1 to 2 (hypermasculine), a IS score equals or approaching to zero, must be regarded as uncertain sex (Ascadi and Nemeskeri, 1970).

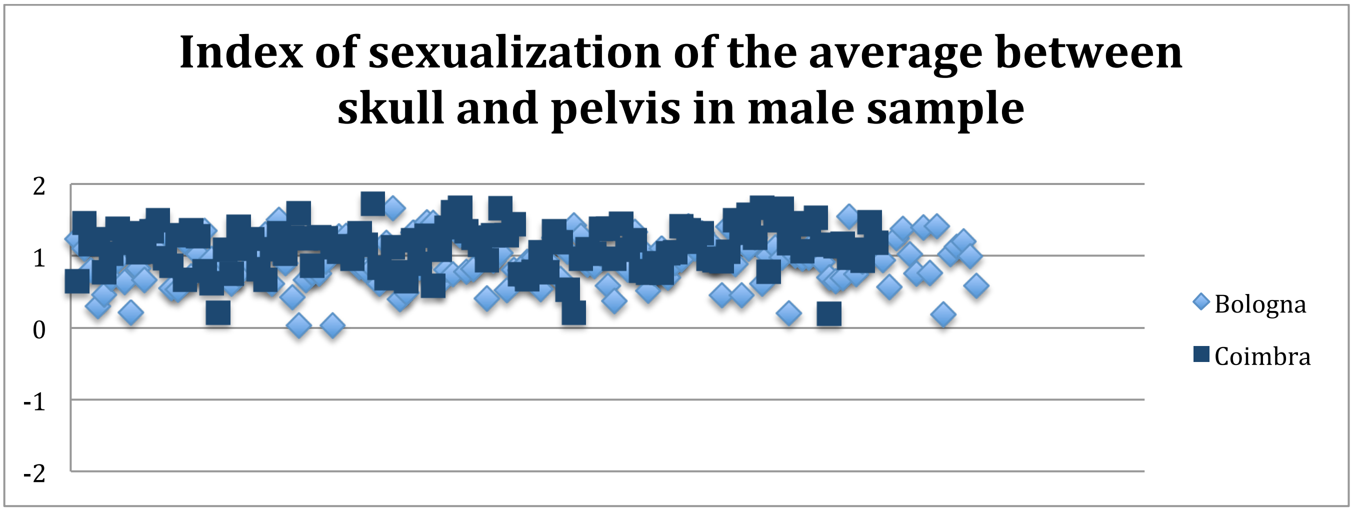
In the following charts (3.28-3.33) each point refers to an individual, y-coordinate represents the value of the coefficient.



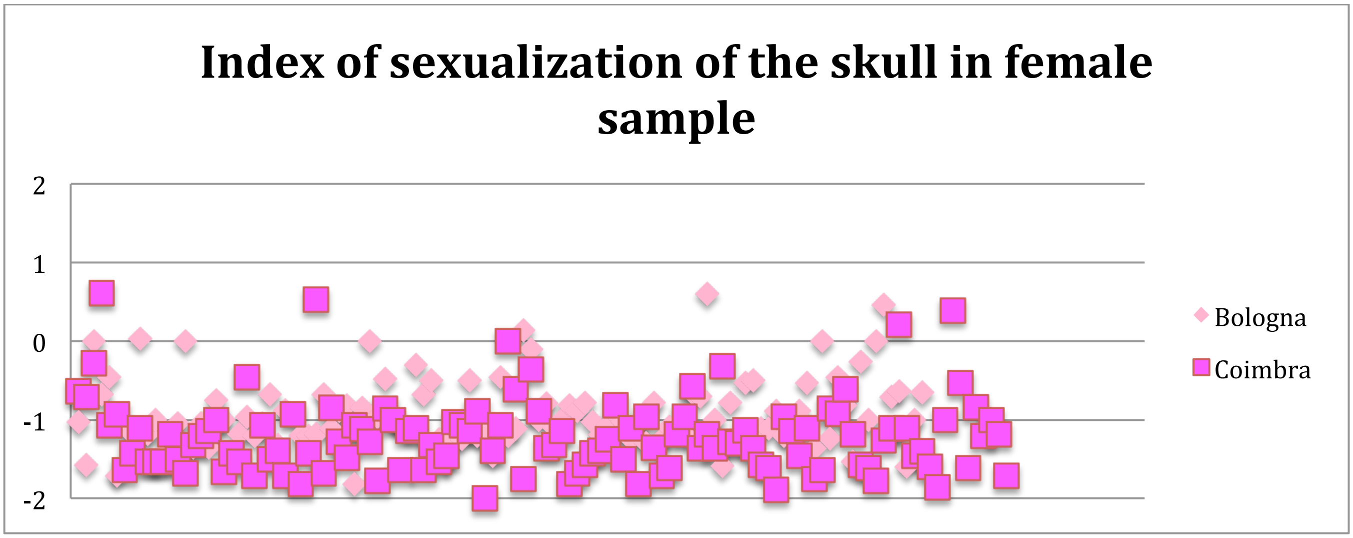
Graph 3.28. Comparison of the skull indices male individuals Bologna-Coimbra



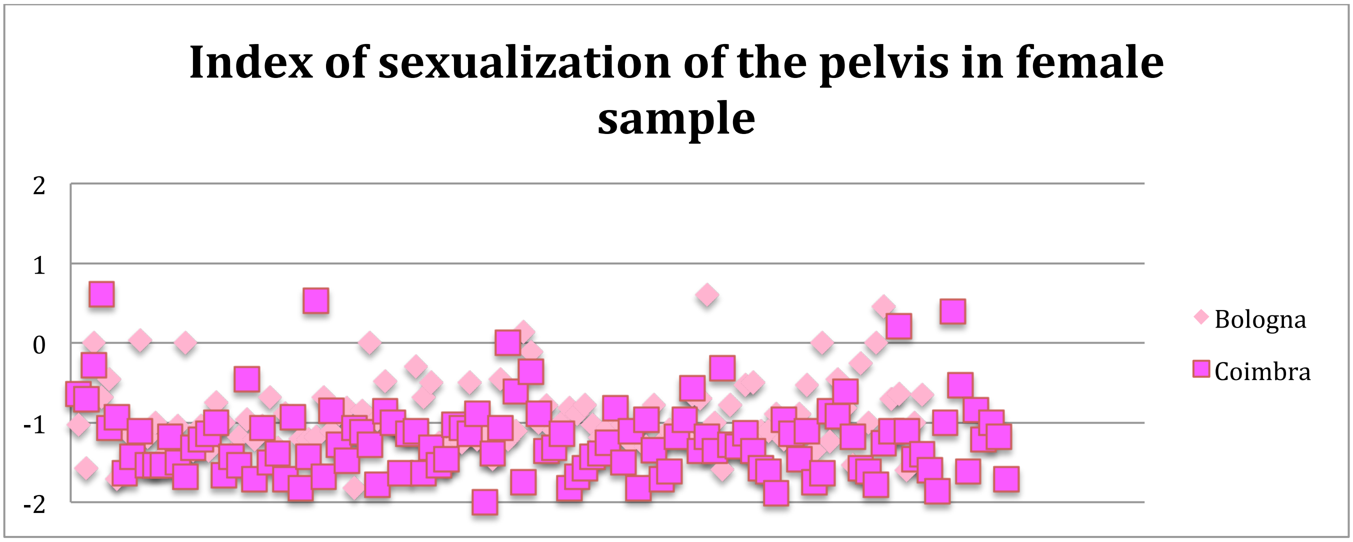
Graph 3.29. Comparison of the pelvis indices male individuals Bologna-Coimbra



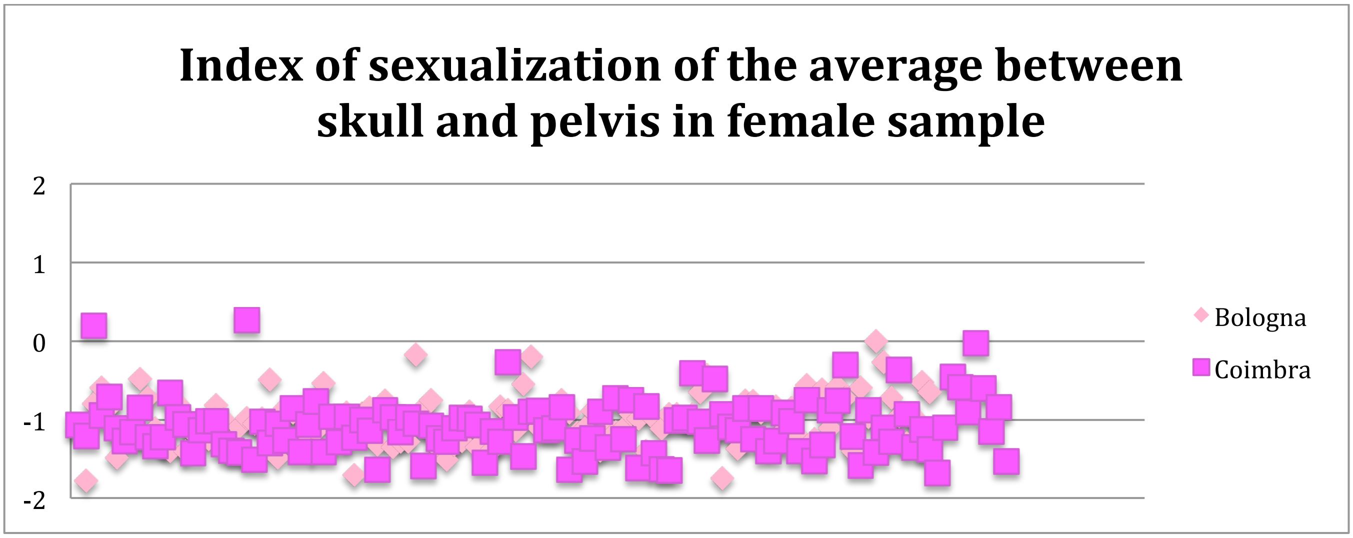
Graph 3.30. Comparison of the skull-pelvis average indices male individuals Bologna-Coimbra



Graph 3.31. Comparison of the skull indices female individuals Bologna-Coimbra



Graph 3.32. Comparison of the pelvis indices female individuals Bologna-Coimbra



Graph 3.33. Comparison of the skull-pelvis average indices female individuals Bologna-Coimbra.

The coefficients values obtained from skull, pelvis, and their average (graphs 3.28-3.33), are more scattered in the sample of Bologna than in the sample of Coimbra, in particular in the male sample.

The coefficient of the skull and pelvis are more scattered than the average, that in both samples gives better results.

The results of the female sample have an higher dispersion than the ones of the male sample, especially for what concern the skull.

The sample of Bologna shows results closer to zero, wich mean less sexualization of the individuals than in Coimbra collection.

- Phenice method

Phenice method was applied bilaterally on the pelvis and the results of each side were compared.
The method confirms the known sex in 87% of the whole sample. The results of the two collections are detailed in table 3.16.

	Ventral Arc				Subpubic Concavity				Ischiopubic Ramus				SEX	
	R		L		R		L		R		L			
MB														
GROUP	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%
M	79	73	65	71	89	84	73	83	78	74	67	77	96	85
F	12	11	11	12	8	8	8	9	9	7	6	7	2	2
0	17	16	16	17	9	9	7	9	20	19	14	16	15	13
TOT	108		92		106		88		106		87		113	
FB														
M	3	4	5	6	1	1	5	7	3	4	4	6	0	0
F	68	87	69	89	75	95	66	89	73	92	65	89	82	95
0	7	9	4	5	3	4	3	4	3	4	4	5	4	5
TOT	78		78		79		74		79		73		86	
MC														
M	50	49	53	52	79	80	76	78	97	95	98	96	90	87
F	10	10	14	14	4	4	4	4	1	1	1	1	1	1
0	41	41	35	34	16	16	18	18	4	4	3	3	13	12
TOT	101		102		99		98		102		102		104	
FC														
M	4	4	2	2	15	15	13	12	6	5	6	5	6	5
F	83	85	88	87	78	71	80	72	89	80	91	81	95	83
O	11	11	11	11	17	15	18	16	16	15	16	14	14	12
TOT	98		101		110		111		111		113		115	

Table 3.16. results of the Phenice method in the two collections

This method is more reliable in the sample of Bologna, in particulary in the females, with 95% of positive match.

The ventral arc, subpubic concavity and ischiopubic ramus show similar percentages of concordance with known among both sides in the whole sample.

In the Bologna sample the subpubic concavity report the higher percentage of confirmation of known sex. In the Coimbra sample instead the ischiopubic ramus report the higher percentage of confirmation of known sex.

The results of the concordance between right and left side do not indicate an increase of reliability of one side of the pelvis compared to the other side (table 3.17).

	Ventral arc		Subpubic concavity		Ischiopubic ramus	
	N.	%	N.	%	N.	%
	MB					
R=L	82	99	77	95	73	92
R≠L	1	1	4	2	6	8
TOT	83		81		79	
	FB					
R=L	66	96	65	94	63	98
R≠L	3	4	4	6	1	2
TOT	69		69		64	
	MC					
R=L	92	94	93	100	97	100
R≠L	6	6	0	-	0	-
TOT	98		93		97	
	FC					
R=L	92	99	104	98	108	100
R≠L	1	1	2	2	0	-
TOT	93		106		108	

Table 3.17. concordance between right and left side in the Phenice method

In the sample of Bologna there is more than 92% of concordance between the right and the left side, the male ischiopubic ramus is the district with the highest number of discrepancies (8%). In the sample of Coimbra the ventral arc provides results which are contrasting in 6% of cases.

- combination of the coefficient of sexualization of Acsadi e Nemerkeri (1970) and the Phenice's method

The coefficient of sexualization of Acsadi e Nemerkeri (1970) and the Phenice's method provided wrong diagnosis in few cases: 8 in the collection of Bologna and 9 in the collection of Coimbra.
Tables 3.18 - 3.19 compare the two tested method (individuals to whom was not possible to apply both of them were omitted).
Individual misdiagnosed by the coefficient of sexualization and corresponding results according to Phenice's method are compared in table 3.18.

IND.*	SEX**	AGE ***	Coefficient of Sexualization			Phenice's Method						
			Skull	Pelvis	Skull+ Pelvis	Ventral Arc		Subpubic Concavity		Ischiopubic Ramus		SEX
						R	L	R	L	R	L	
BOLOGNA												
5	M	40	0,96	-0,35	0,3	F	M	M	M	M	M	M
15	M	77	-0,03	1,36	0,66	NR	M	NR	M	NR	M	M
38	M	77	-0,15	1	0,42	M	M	M	M	0	0	M
60	M	66	-0,46	1,25	0,40	M	M	M	M	M	M	M
72	M	59	-0,03	1,6	0,78	0	0	0	0	0	0	0
133	M	19	-0,25	0,65	0,2	M	NR	F	NR	0	NR	0
67	F	67	0,14	-1,25	-0,55	F	F	F	F	M	M	F
100	F	63	0,6	-1,47	-0,43	F	NR	F	NR	F	NR	F
COIMBRA												
45	M	21	-0,32	1,65	0,66	M	M	M	M	M	M	M
60	M	48	-0,18	0,6	0,21	M	M	M	M	NR	M	M
257	M	57	-0,68	1,06	0,19	0	M	M	NR	0	0	0
9	F	48	-0,28	0,68	0,19	F	F	F	F	F	F	F
10	F	21	0,61	-1,3	-0,95	NR	NR	F	F	F	F	F
49	F	74	-0,46	1	0,27	0	0	0	0	M	M	0
76	F	45	0,53	-1	-0,77	F	F	F	F	F	F	F
272	F	91	0,21	-0,95	-0,37	F	F	0	0	F	F	F
291	F	52	0,39	-1,3	-0,46	F	F	M	M	M	M	M

Table 3.18. Wrong values of coefficient of sexualization and values of Phenice's method
* the number identifies the ID number of individuals of both collections and it is referred to the number of the wood box.,
** sex know, *** age know

In the coefficient of sexualization the average of values generally confirms the know sex in spite of skull and pelvis providing a wrong diagnosis if considered separately (fig. 3.2.4) (except two cases in Coimbra's collection).

Figure 3.2.4 a-b) skull and right coxal bone of male n.15 c-d-e) skull and coxal bones of female n.100 of Bologna

In the sample of Bologna the combination of the two methods allowed for a correct diagnosis in 6 cases out of 8 where some values of skull and pelvis lead to misdiagnosis.

In the sample of Coimbra the combination of the two methods allowed for a correct diagnosis in 6 cases out of 9 where some values of skull and pelvis lead to misdiagnosis. For two individuals (N. 291, figure 3.2.5 e N. 9, figure 3.2.6 a) the two methods did not allow a correct sexing. In three individuals (N.133, N.257, N.49) the results obtained by both methods do not give a correct diagnosis as the score points toward an indeterminated diagnosis.

Figure 3.2.5 skull and right coxal bone of the female n.291 of the collection of Coimbra: the skull show masculine traits in glabella, orbital shape and jaw; the right coxal bone show femenin traits when calculating the coefficient of sexualization but when applying the Phenice method the morphology of right and left subpubiv concavity and ischiopubic ramus is masculine.

Figure 3.2.6 a) left coxal bone of the female n.9 of the collection of Coimbra; b) left coxal bone of the female n.52 of the collection of Bologna c) left coxal bone of the female n.122 of the collection of Bologna; the three coxal bons belong to females individuals but the different morphology of the great sciatic notch is markedly different.

The detailed values of coefficient of sexualization and wrong values of Phenice's method are reported in table 1.19 in order to compare the results. In the male sample the coefficient of sexualization will confirm the know sex if the Phenice's method provides a wrong diagnosis.

The method of Phenice is more reliable in the sample of Bologna, giving only two misdiagnosis (two males sexed as females).

In the collection of Coimbra the method of Phenice is less reliable in the female group with 6 wrong diagnosis; in the male sample only one individual was sexed uncorrectly.

IND.*	SEX**	AGE***	Coefficent of Sexualization			Phenice's Method						
			Skull	Pelvis	Skull+ Pelvis	Ventral Arc		Subpubic Concavity		Ischiopubic Ramus		SEX
						R	L	R	R	L	R	
BOLOGNA												
93	M	44	1,17	0,95	1,06	F	F	0	0	F	F	F
161	M	50	0,32	0,05	0,18	F	F	F	F	NR	NR	F
COIMBRA												
61	M	55	1,07	1,1	1,08	F	F	F	F	M	M	F
30	F	35	-1,32	-1,55	-1,43	F	F	M	M	F	F	M
89	F	83	-1,48	-0,45	-0,96	F	F	M	M	M	M	M
147	F	40	0	-0,55	-0,27	0	0	M	M	M	M	M
155	F	52	-0,89	-0,9	-0,89	F	NR	M	M	M	M	M
233	F	79	-1,11	-0,4	-0,76	M	M	M	M	M	M	M
291	F	52	0,39	-1,3	-0,46	F	F	M	M	M	M	M

Table 1.19. Values of coefficient of sexualization and wrong values of Phenice's method.

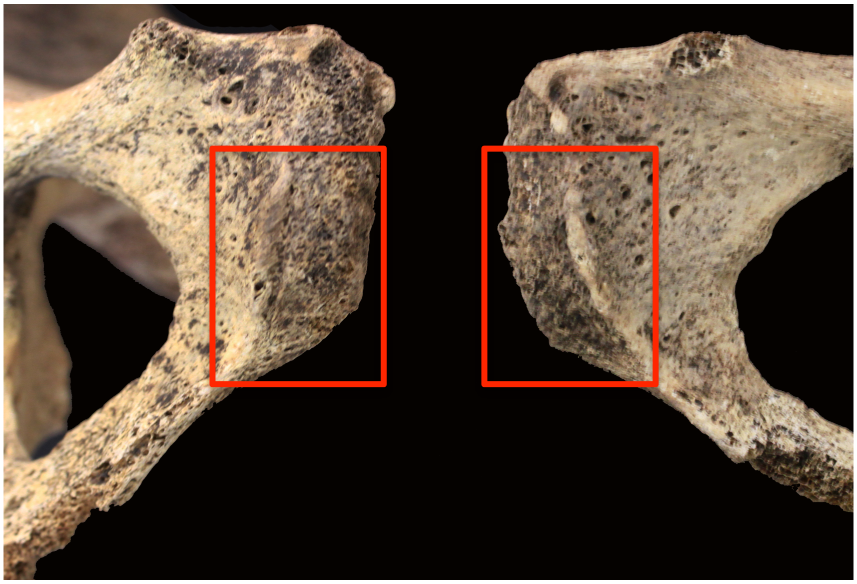


Figure 3.2.7 coxal bones of the male n.161 of the collection of Bologna. It can be noticed the presence of the ventral arc athough the individual is male.

CHAPTER 4: Comparisons

4.1) Comparisons between the results of the age estimation of this study and similar researches on identified populations

There are many studies on the reliability of the methods tested in this study on other identified collections. The results of this study are compared with similar studies (table 4.1).

Collection	Location	Period	Sample	Method/methods tested	Autor
Collection of Sassari	Museum of Anthropology at the University of Bologna	first half of the twentieth century	404: 200M-204F	Suchey-Brooks (1990), Lovejoy et al. (1985)	Hens, 2008
Collections of the Laboratory of Forensic Anthropology and osteological	University of Antioquia in Medellin, Colombia	present day	110:81M-29F	Suchey-Brooks (1990), Lovejoy et al. (1985)	Rivera - Sandoval, 2014
Collection of the Department of Anatomy	University of Chiang Mai, Thailand	collected since 1990*	66: 37M-29F	Suchey-Brooks (1990), Lovejoy et al. (1985)	Schmitt, 2004
Canadian population of the nineteenth century, from the cemetery of St. Thomas Anglican Church in Belleville	Ontario	1821-1874	49**	Suchey-Brooks (1990), Lovejoy et al. (1985), Meindl and Lovejoy (1985)	Saunders et al . 1992
Collection of the Department of Anatomy	University Museum, University of Tokyo	collected between 1885 and 1944*	416:326M-90F	Suchey-Brooks (1990)	Sakaue, 2006
Collection of Coimbra	Departamento de Ciências da Vida, Universidade de Coimbra	nineteenth century - first half of the twentieth century	215:105M-110F	Lovejoy et al. (1985)	Santos, 1992
Collection of Sassari, Collection of Coimbra	Museum of Anthropology at the University of Bologna,Departamento de Ciências da Vida, Universidade de Coimbra	first half of the twentieth century	904: 268M-218F SS, 227M-191F CMB	Belcastro (2008)	Belcastro, 2008

Table 4.1 . comparison between this study and similar studies.
*the period of the collection is not specified ** the sex suddivision is not specified

All these studies identified a general trend to higher values of bias and inaccuracy with increasing age and the know age tends to be underestimated.

- collection of Sassari Museum of Anthropology at the University of Bologna (Hens, 2008)

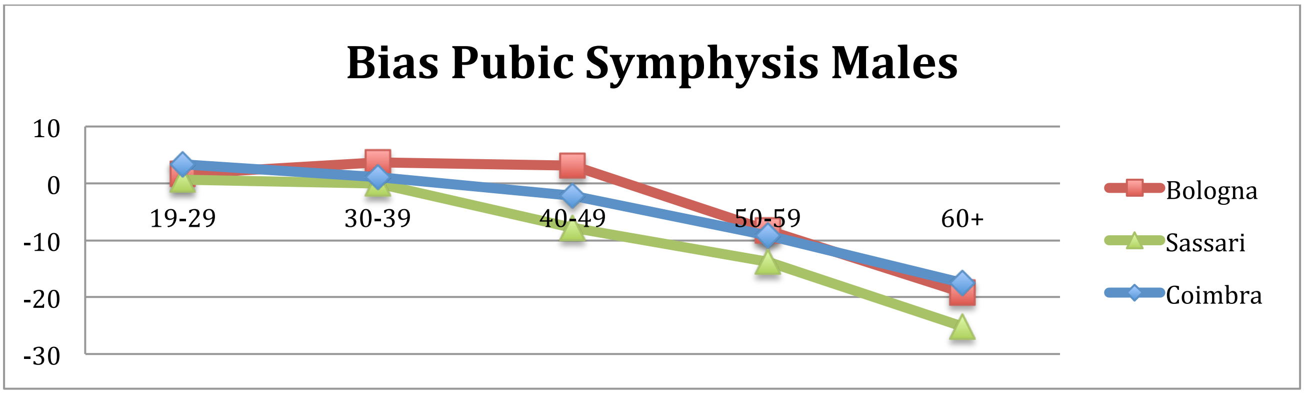
This study tested the Suchey-Brooks pubic symphysis age estimation method and the auricular surface method developed by Lovejoy et al. on a sample of 404 adult individuals of the Sassari Collection, owned by the Museum of Anthropology of the University of Bologna. The sample is composed by 204 males (aged 18–86) and 200 females (aged 17–98) with known sex and age. The reliability for both methods was tested using standard measures of bias and inaccuracy. The index of bias and inaccuracy obtained from Hens (2008) on the collection of Sassari (table 4.2) don't show big differences. The index of the three samples are compared in graphs 4.1 - 4.8 which show a very similar trend for the values of bias and inaccuracy in the methods of the pubic symphysis and auricular surface,.

The method of the pubic symphysis tends to be more reliable between 17-29 years, and in both sexes after 40 years the age tends to be underestimated; also the auricular surface shows the tendency to underestimate the age after 39 years.

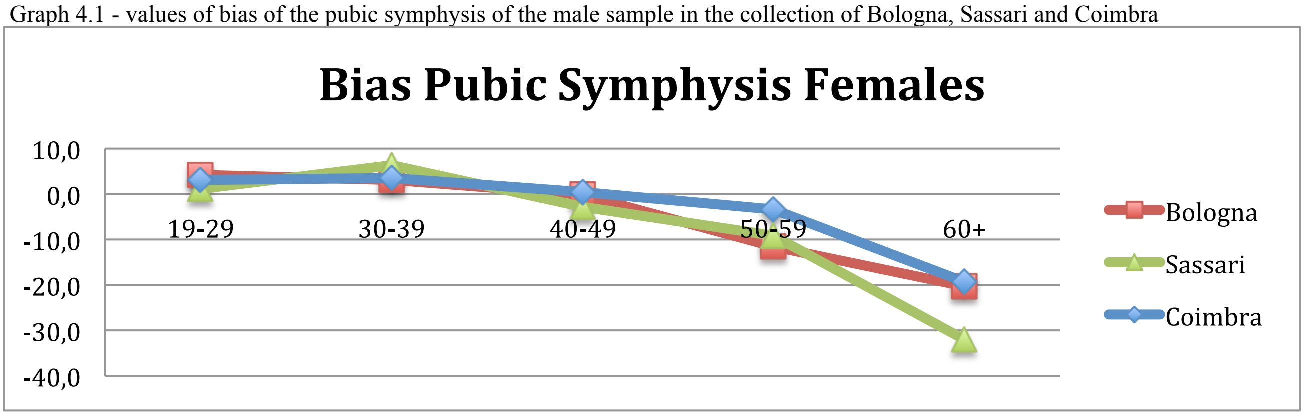
Only the values of bias of the pubic symphysis of the male sample show big differences between 40-49 years (graph 4.1).

	MALE							FEMALE					
	BIAS				INACCURACY			BIAS				INACCURACY	
	N.	Pubic symphysis	N.	Auricular surface	Pubic symphysis	Auricular surface		N.	Pubic symphysis	N.	Auricular surface	Pubic symphysis	Auricular surface
	SASSARI (Hens, 2008)												
18-29	36	0,6	38	3,5	4,9	4,4	18-29	51	1,1	57	3,8	4	4,6
30-39	34	0	35	1,3	7,3	5,4	30-39	36	6,3	40	3,2	10,5	7,4
40-49	37	-7,8	39	-4,1	8,9	6,5	40-49	36	-2,8	37	-5,9	8,2	9,1
50-59	37	-13,8	38	-12,3	14,4	12,3	50-59	19	-9,1	20	-12,5	12,3	15,4
60+	58	-25,2	54	-22,8	25,2	23,1	60+	46	-32	46	-29,3	32	29,3
	BOLOGNA												
18-29	20	1,66	25	7,82	4,1	7,9	18-29	17	4,2	23	5,9	5,2	6,1
31-39	16	3,65	19	1,13	6,2	4,6	30-39	18	3,1	21	-0,6	5,3	1,9
40-49	13	3,1	16	-1,4	6,6	6,8	40-49	9	-0,2	12	-1,3	5,2	4,6
50-59	1	-8,25	22	-9,4	9,8	10,6	50-59	9	-11,5	14	-4,5	11,5	5,8
60+	43	-19,2	41	-19,7	19,1	19,3	60+	28	-20,3	40	-18,4	20,3	18,3
	COIMBRA												
20-29	16	3,4	21	7,5	4,2	7,8	19-29	14	3,1	16	10,3	4	10,3
30-39	19	1,1	24	0,3	3,7	2,6	30-39	18	3,5	19	2,7	5	4,5
40-49	20	-2,1	23	-1,9	4,1	5	40-49	13	0,4	15	-2,1	2,3	6,2
50-59	21	-9,2	22	-6,2	11,1	6,5	50-59	19	-3,4	21	-5,4	5,8	6,8
60+	26	-17,5	23	-17,2	17,7	17,2	60+	45	-19,4	46	-20	19,4	20

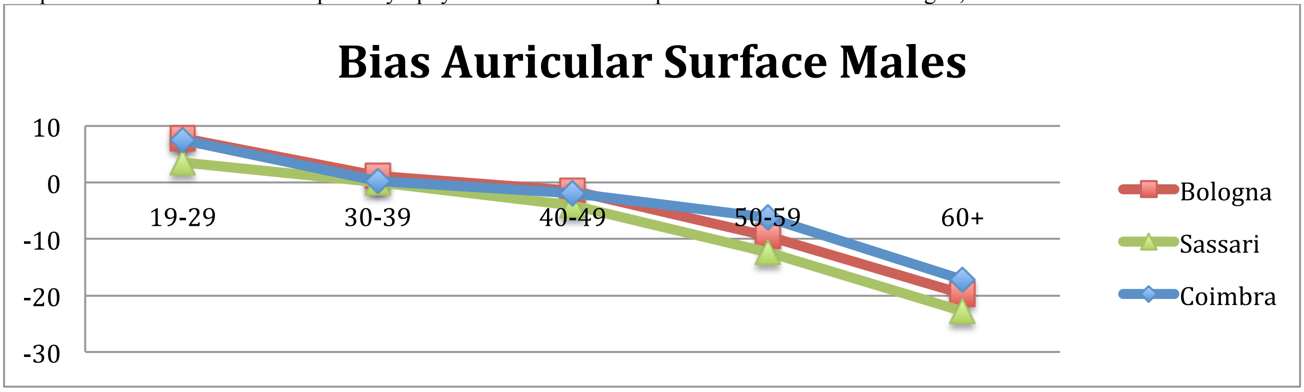
Table 4.2. index of bias and inaccuracy of the study on the collection of Sassari (Hens, Belcastro, 2008), and the index on the collection of Bologna and Coimbra (present study)



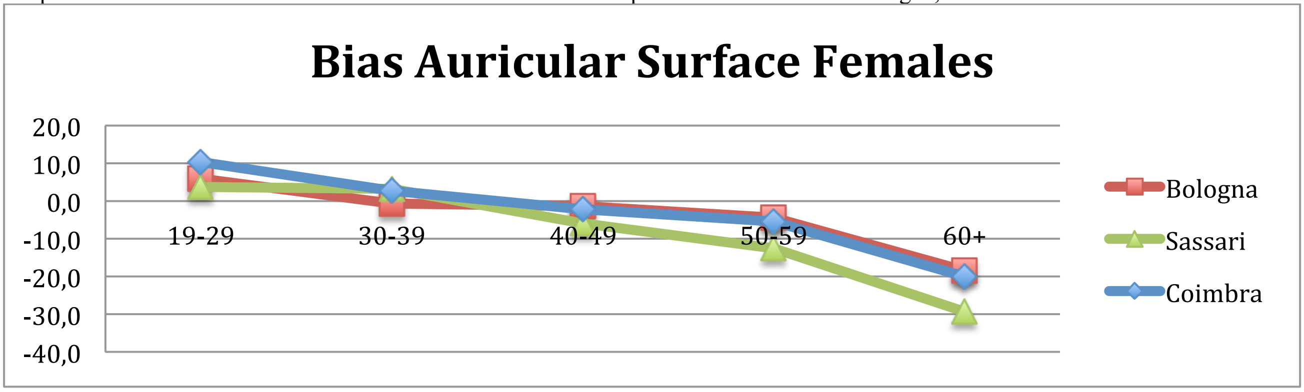
Graph 4.1 - values of bias of the pubic symphysis of the male sample in the collection of Bologna, Sassari and Coimbra



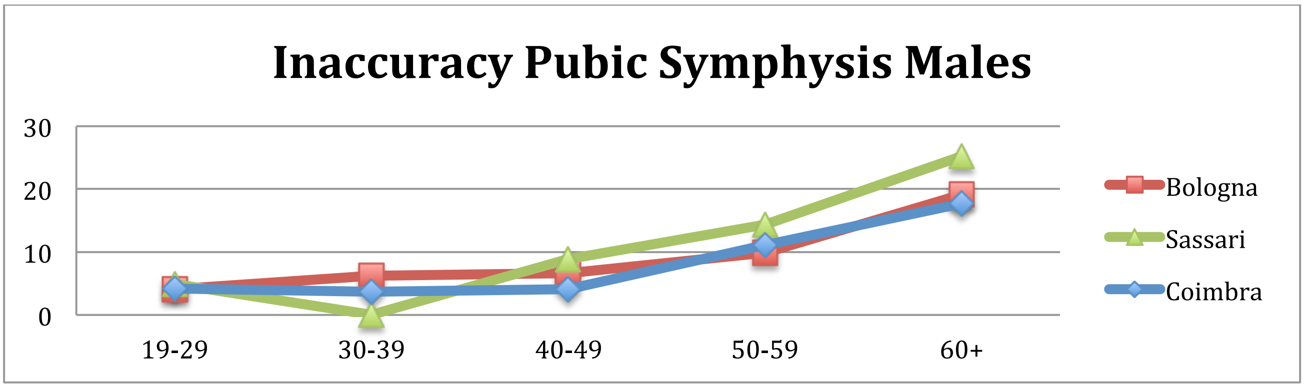
Graph 4.2 . values of bias of the pubic symphysis of the female sample in the collection of Bologna, Sassari and Coimbra



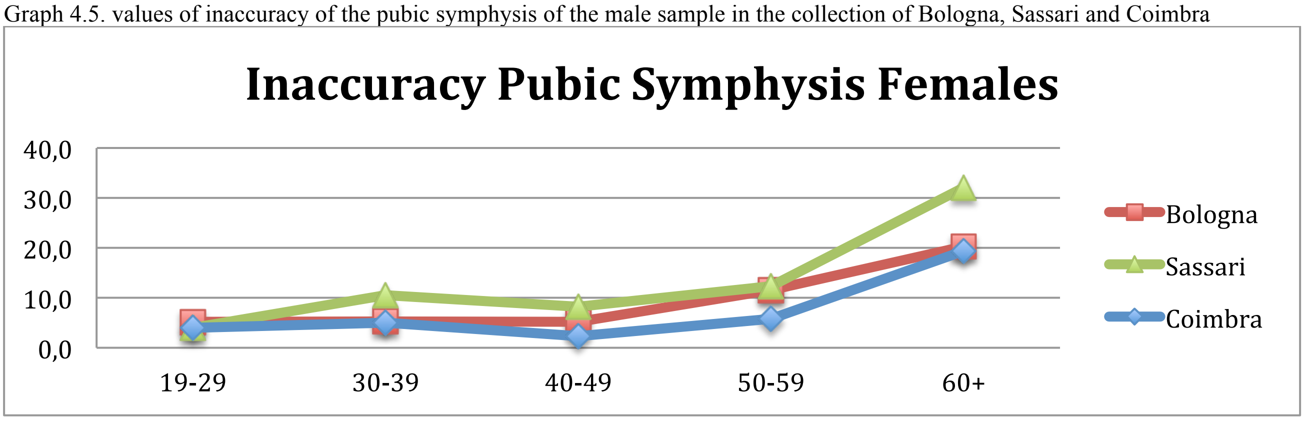
Graph 4.3. values of bias of the auricular surface of the male sample in the collection of Bologna, Sassari and Coimbra



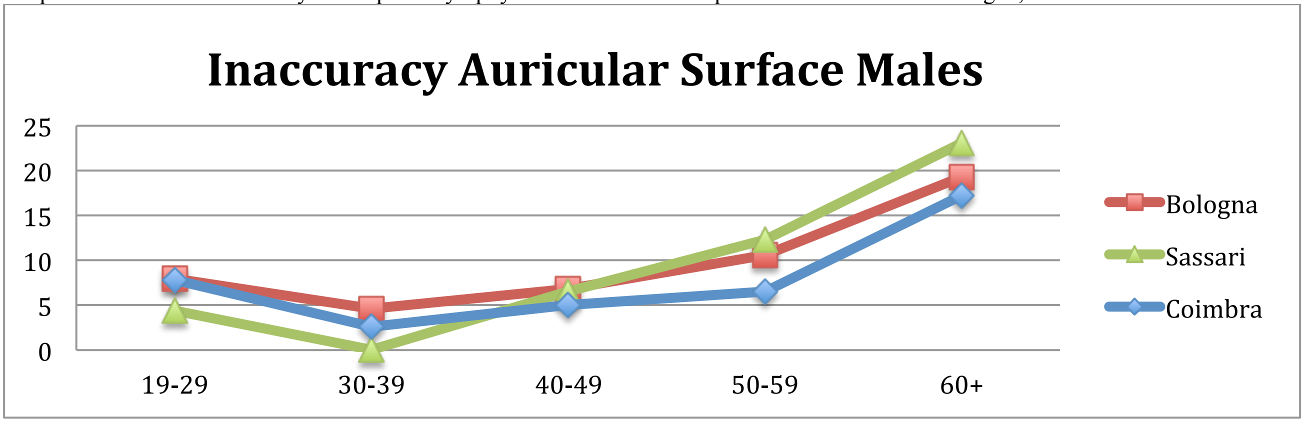
Graph 4.4. values of bias of the auricular surface of the female sample in the collection of Bologna, Sassari and Coimbra



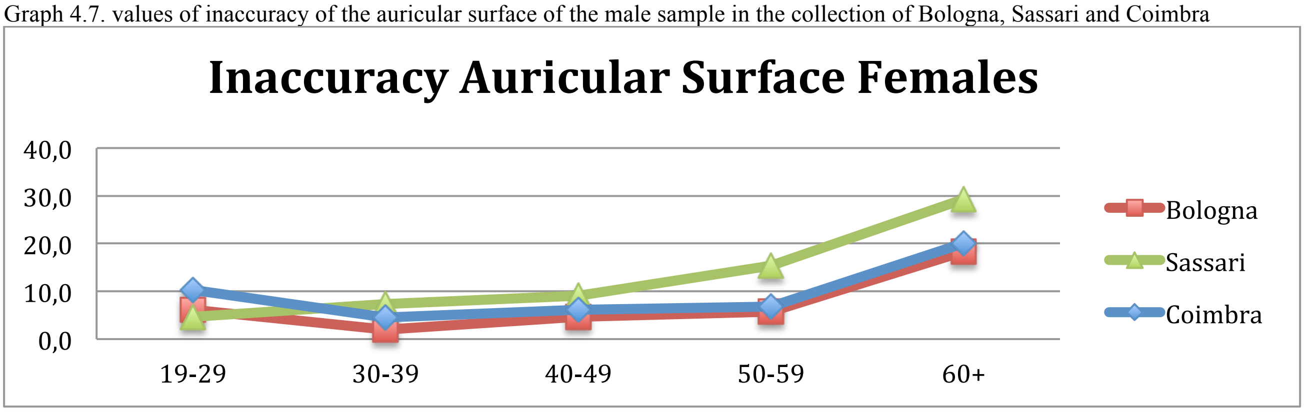
Graph 4.5. values of inaccuracy of the pubic symphysis of the male sample in the collection of Bologna, Sassari and Coimbra



Graph 4.6. values of inaccuracy of the pubic symphysis of the female sample in the collection of Bologna, Sassari and Coimbra



Graph 4.7. values of inaccuracy of the auricular surface of the male sample in the collection of Bologna, Sassari and Coimbra



Graph 4.8. values of inaccuracy of the auricular surface of the female sample in the collection of Bologna, Sassari and Coimbra

- collections of the Laboratory of Forensic Anthropology and osteological (University of Antioquia in Medellin, Colombia; Rivera - Sandoval, 2014)

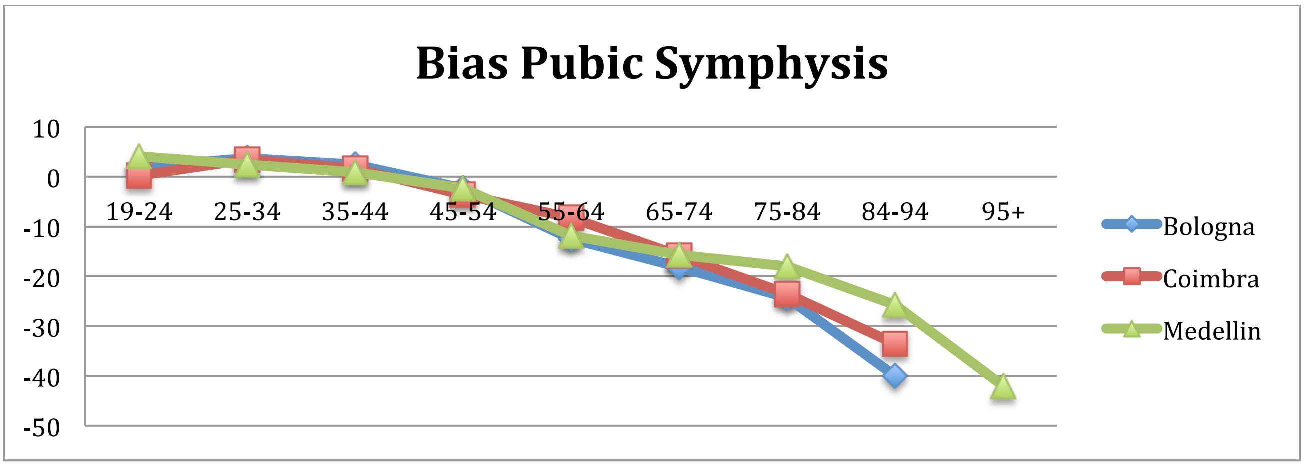
This study was conduced on a sample of 110 adult individuals (81 males, 29 females) of two contemporary collections wich came from Universal e San Pedro cemetery of Antioquia with known sex and age. Reliability for both methods was tested using standard measures of bias and inaccuracy. The index of bias and inaccuracy obtained by Rivera-Sandoval (2014) on the collection of Antioquia (Medellin, Colombia) show a tendency to increase with increasing age for both methods (tables 4.3) (the values of bias and inaccuracy of the present study were reorganized by joining the male and female sample and re-formulating ages range to compare the results of the two studies).

	BIAS				INACCURACY	
	N.	Pubic symphysis	N.	Auricular surface	Pubic symphysis	Auricular surface
AGE RANGE	MEDELLIN M+F					
19-24	6	4,08	17	1,69	4,08	3,28
25-34	7	2,43	14	2,31	3	3,19
35-44	6	0,92	10	-1,54	4,46	5,88
45-54	1	-2,3	5	-4,07	7,3	10,63
55-64	1	-11,91	6	-12,7	11,91	12,92
65-74	7	-15,73	6	-20,85	15,73	20,85
75-84	9	-18,04	7	-24,92	18,04	24,92
84-94	3	-25,67	3	-30,67	25,67	30,67
95+	1	-42	1	-44,6	42	44,6
TOT	41		69			
	BOLOGNA M+F					
19-24	16	1,9	24	8,4	2,8	8,5
25-34	35	3,7	41	3,3	6,6	4,2
35-44	27	2,4	34	-1,4	4,4	4,5
45-54	23	-2,4	29	-3,2	7,6	7
55-64	33	-12,5	42	-10,2	12,8	10,5
65-74	32	-18,2	37	-17,1	18,2	17,1
75-84	30	-24,3	21	-19,2	24,3	19,2
84-94	4	-40	2	-32,6	40	32,6
95+	0	-	0	-	-	-
TOT	200		230			
	COIMBRA M+F					
19-24	9	0,2	11	9,9	2,4	9,9
25-34	37	3,4	45	5,3	4,1	5,7
35-44	36	1,7	42	0,5	4,7	4,7
45-54	39	-3,6	43	-3,5	5,3	5
55-64	32	-8,2	34	-8,7	9,2	8,9
65-74	29	-15,8	27	-16,5	15,8	16,5
75-84	22	-23,5	22	-18,1	23,5	18,1
84-94	7	-33,6	7	-29,7	33,6	29,7
95+	0	-	0	-	-	-
TOT	211		231			

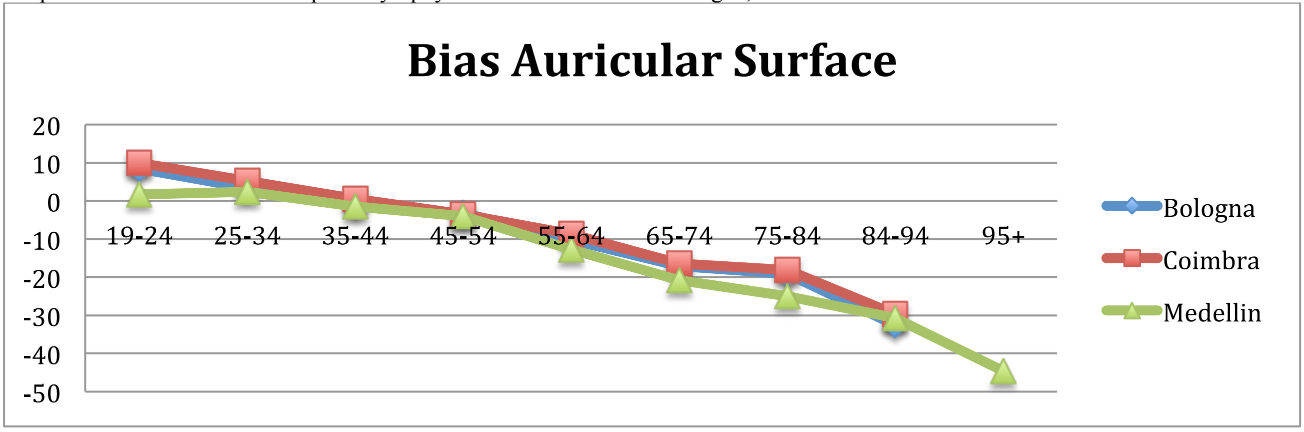
Table 4.3 index of bias and inaccuracy of pubic symphysis and auricular surface obtained by Rivera-Sandoval (2014) compared with this study, the values of bias and inaccuracy were reorganized by joining the male and female sample and re-formulating ages to compare the results of the two studies.

The comparison of the results of the two studies of the pubic symphysis shows that the values of bias and inaccuracy are higher in sample of Medellin in the age range 45 to 84 years compared to those obtained in Bologna and Coimbra samples. The values of auricular surface are generally lower than the Medellin sample.

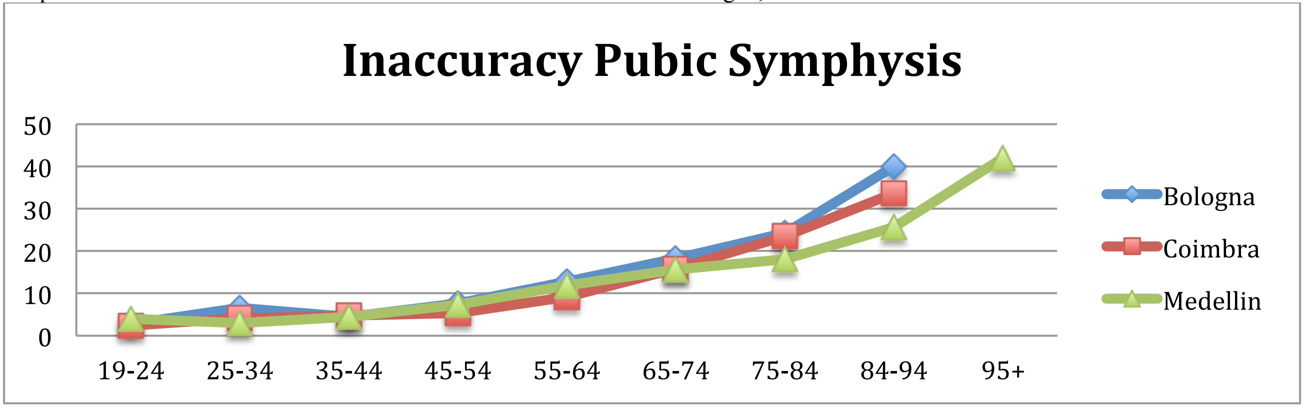
The values of bias and inaccuracy of 3 samples are represented in the graphs 4.9 – 4.12.



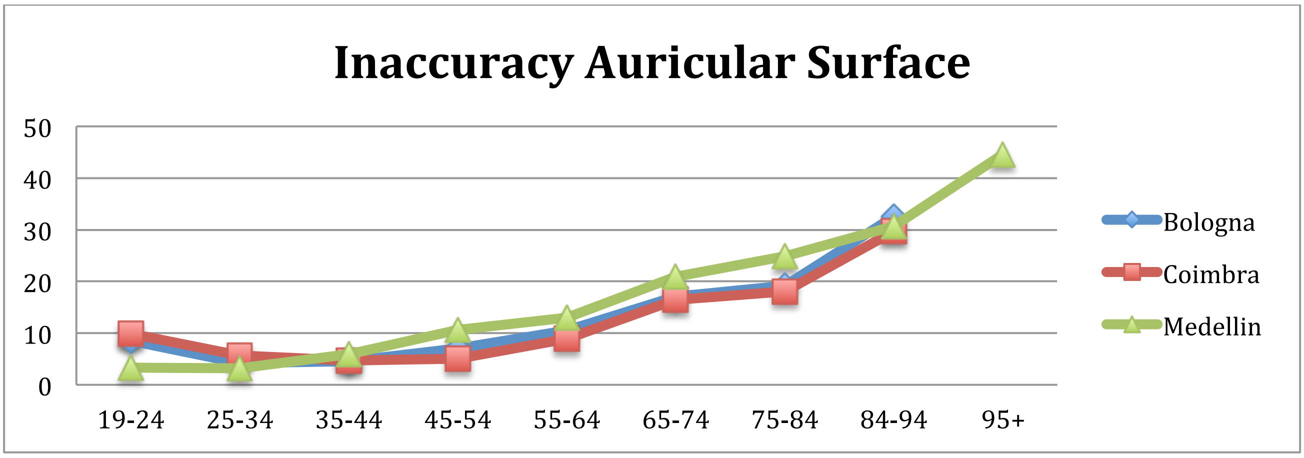
Graph 4.9. values of bias of the pubic symphysis in the collection of Bologna, Coimbra and Medellin



Graph 4.10. values of bias of the auricular surface in the collection of Bologna, Coimbra and Medellin



Graph 4.11. values of inaccuracy of the pubic symphysis in the collection of Bologna, Coimbra and Medellin



Graph 4.12. values of inaccuracy of the auricular surface in the collection of Bologna, Coimbra and Medellin

- the collection of the Department of Anatomy (University of Chiang Mai, Thailand; Schmitt, 2004)

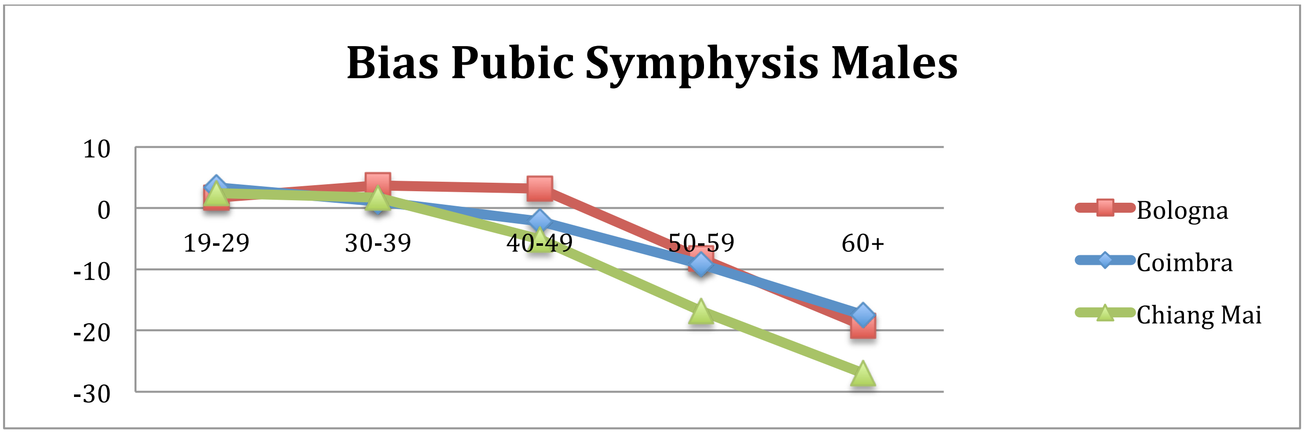
Schmitt (2004) tested for the first time the method of Suchey-Brooks and the method of Lovejoy on a Thai population of 65 individuals (collection of the Department of Anatomy). He found an increase of the values of bias and inaccuracy with increasing age and the known age tends to be underestimated (table 4.4).

	MALE							FEMALE					
	BIAS				INACCURACY			BIAS				INACCURACY	
AGE RANGE	N.	Pubic symphysis	N.	Auricular surface	Pubic symphysis	Auricular surface		N.	Pubic symphysis	N.	Auricular surface	Pubic symphysis	Auricular surface
	CHIANG MAI (Schmitt, 2004)												
20-29	2	2,4	2	2	2,4	2	20-29	0	-	0	-	-	-
30-39	3	1,7	3	-7,3	16,5	9,3	30-39	5	1,5	5	-6,3	6,7	6,3
40-49	10	-5,1	10	-7,5	8	8,3	40-49	7	-4,8	8	-14,1	9,7	14,1
50-59	11	-16,8	10	-17,6	16,8	17,6	50-59	6	-17,2	6	-21,9	17,2	21,9
60+	11	-27	11	-31,9	27,2	31,9	60+	10	-32,2	10	-30,4	32,2	30,4
	BOLOGNA												
18-29	20	1,66	25	7,82	4,1	7,9	18-29	17	4,2	23	5,9	5,2	6,1
31-39	16	3,65	19	1,13	6,2	4,6	30-39	18	3,1	21	-0,6	5,3	1,9
40-49	13	3,1	16	-1,4	6,6	6,8	40-49	9	-0,2	12	-1,3	5,2	4,6
50-59	1	-8,25	22	-9,4	9,8	10,6	50-59	9	-11,5	14	-4,5	11,5	5,8
60+	43	-19,2	41	-19,7	19,1	19,3	60+	28	-20,3	40	-18,4	20,3	18,3
	COIMBRA												
20-29	16	3,4	21	7,5	4,2	7,8	19-29	14	3,1	16	10,3	4	10,3
30-39	19	1,1	24	0,3	3,7	2,6	30-39	18	3,5	19	2,7	5	4,5
40-49	20	-2,1	23	-1,9	4,1	5	40-49	13	0,4	15	-2,1	2,3	6,2
50-59	21	-9,2	22	-6,2	11,1	6,5	50-59	19	-3,4	21	-5,4	5,8	6,8
60+	26	-17,5	23	-17,2	17,7	17,2	60+	45	-19,4	46	-20	19,4	20

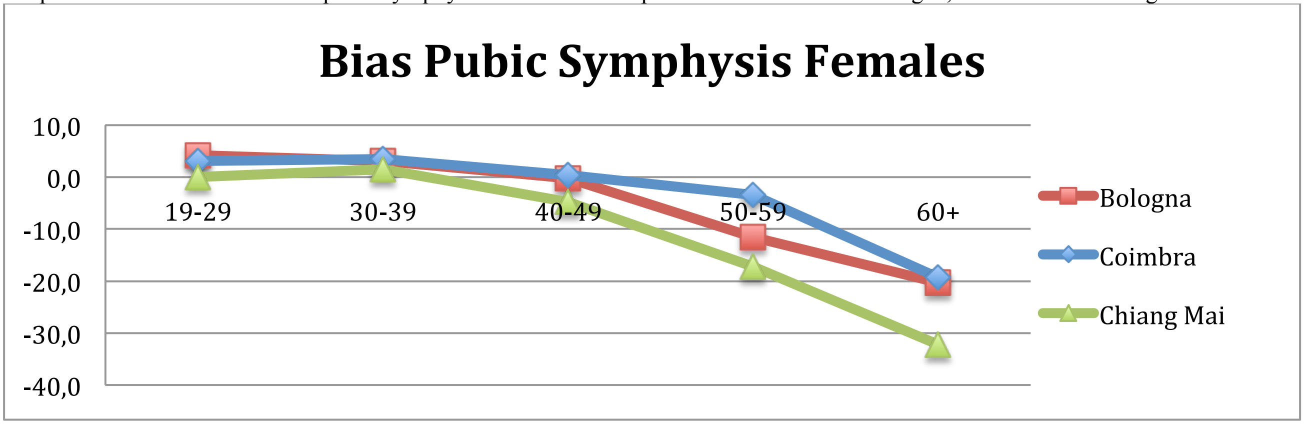
Table 4.4. values of bias and inaccuracy obteined by Schmitt (2004) and the results of this study

From the comparison of the results obtained by Schmitt (graphs 4.13 - 4.20) we can see that generally the values of bias underestimate the known age. The values of inaccuracy of the sample of Chiang Mai are higher in both the male and female samples than those obtained in Bologna and Coimbra samples, in particular over 40 years.

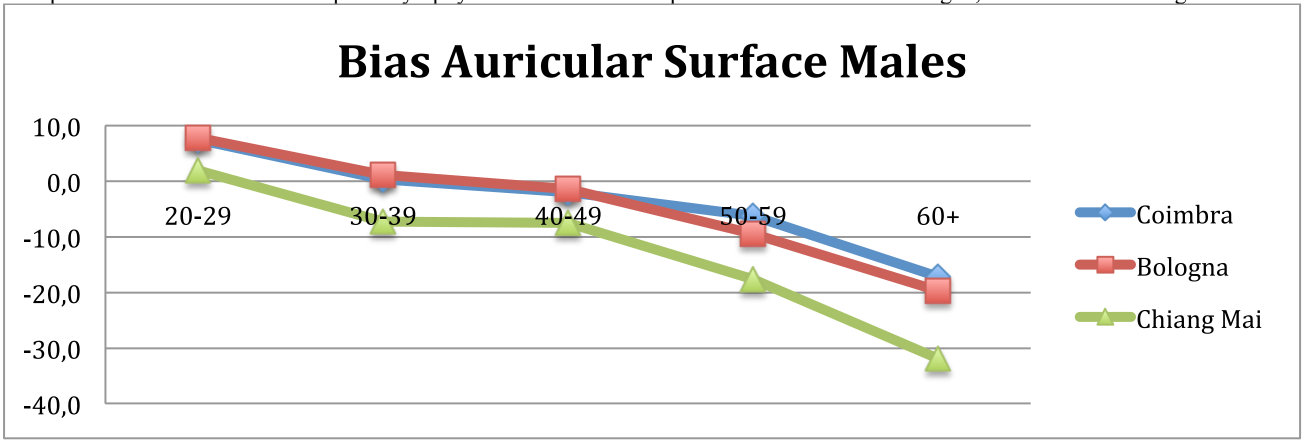
The values of bias and inaccuracy of 3 samples are represented in the graphs 4.13 - 4.20.



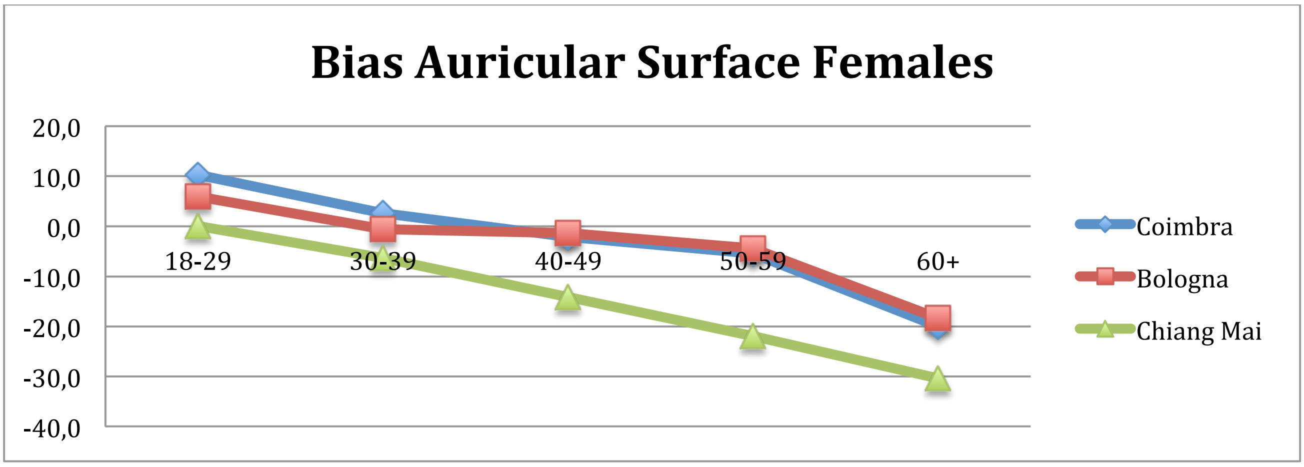
Graph 4.13. values of bias of the pubic symphysis in the male sample of the collection of Bologna, Coimbra and Chiang Mai



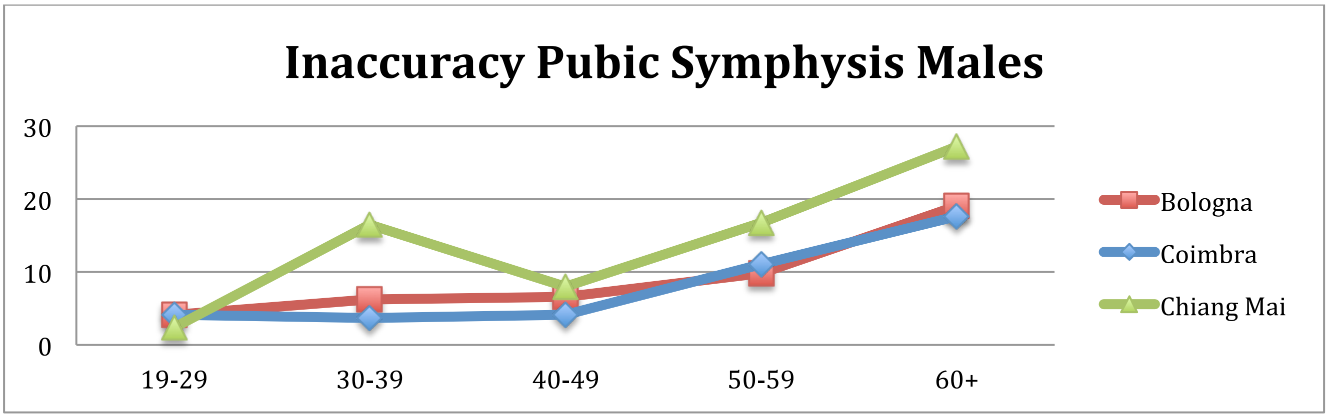
Graph 4.14. values of bias of the pubic symphysis in the female sample of the collection of Bologna, Coimbra and Chiang Mai



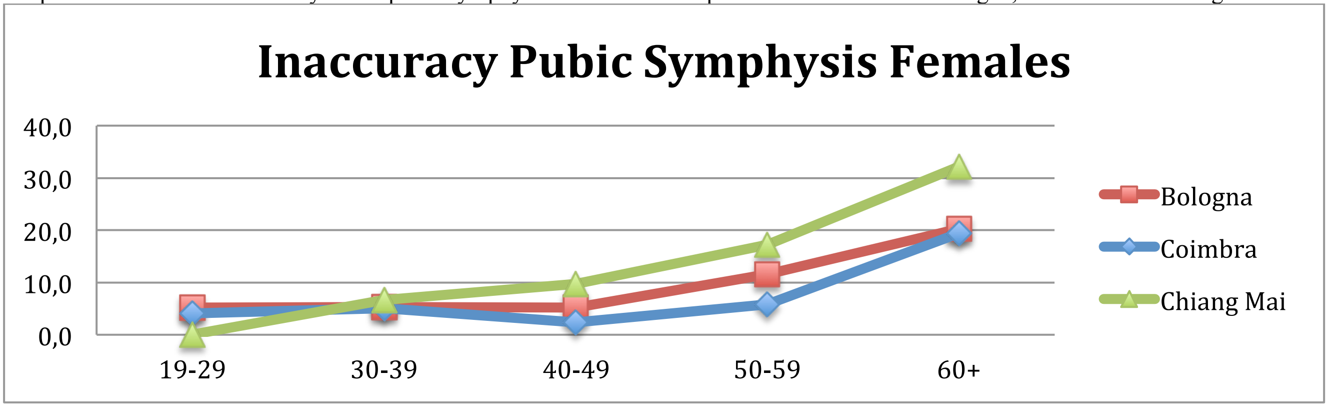
Graph 4.15. values of bias of the auricular surface in the male sample of the collection of Bologna, Coimbra and Chiang Mai



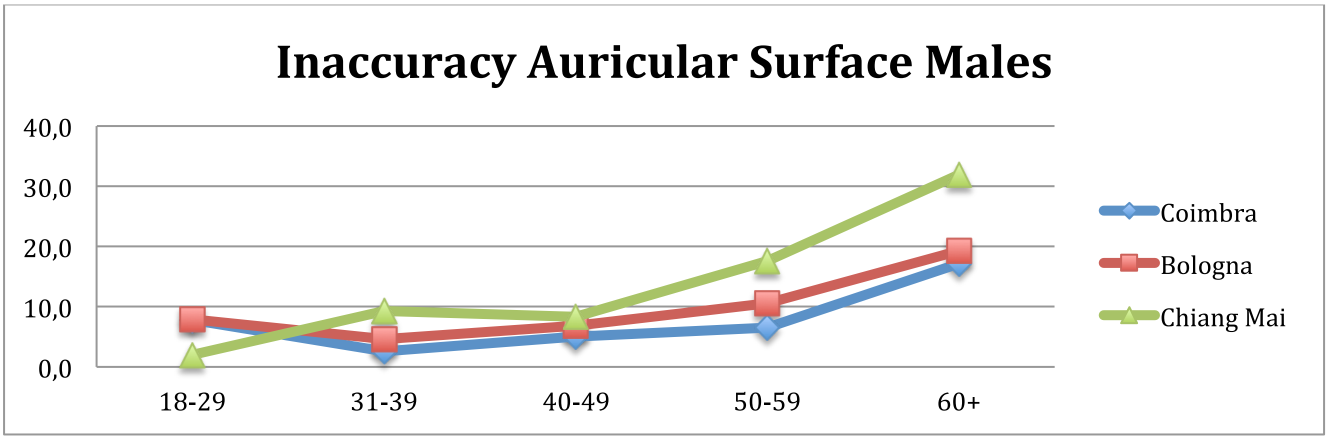
Graph 4.16. values of bias of the auricular surface in the female sample of the collection of Bologna, Coimbra and Chiang Mai



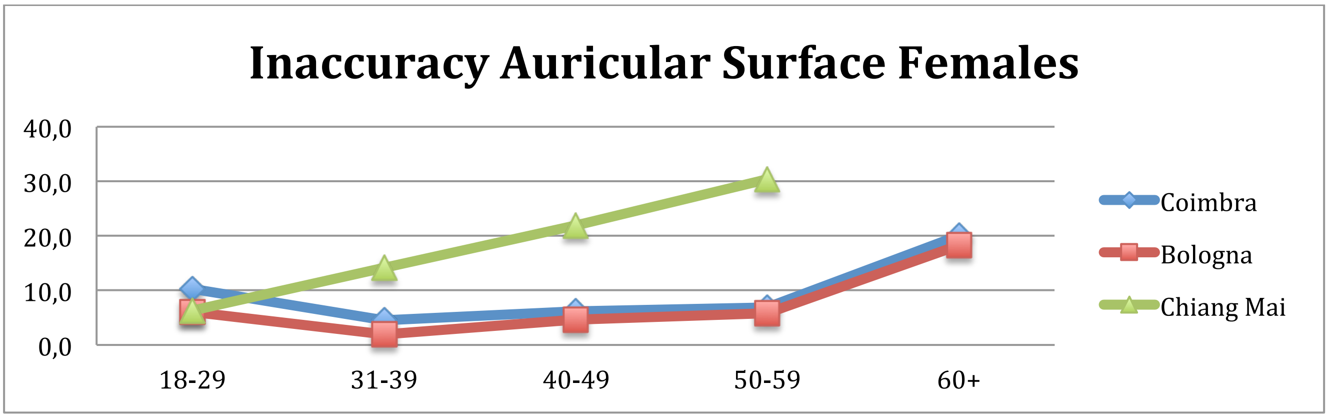
Graph 4.17. values of inaccuracy of the pubic symphysis in the male sample of the collection of Bologna, Coimbra and Chiang Mai



Graph 4.18. values of inaccuracy of the pubic symphysis in the female sample of the collection of Bologna, Coimbra and Chiang Mai



Graph 4.19. values of inaccuracy of the auricular surface in the male sample of the collection of Bologna, Coimbra and Chiang Mai



Graph 4.20. values of inaccuracy of the auricular surface in the female sample of the collection of Bologna, Coimbra and Chiang Mai

- a Canadian population of the nineteenth century, from the cemetery of St. Thomas Anglican Church in Belleville, (Ontario; Saunders et al. 1992)

Saunders et al. (1992) tested all the methods used in this study (Suchey and Brooks (1990), Lovejoy et al. (1985), Iscan et al., (1984, 1985, 1986) and Meindl and Lovejoy, (1985)) on a Canadian population of the nineteenth century (from the cemetery of St. Thomas Anglican Church in Belleville, Ontario). The values of bias and inaccuracy were reorganized by combining the male and female samples in order to compare the results of the two studies (table 4.5).

	BIAS								INACCURACY			
	N.	Pubic Symphysis	N.	Auricular Surface	N.	Fourth Rib	N.	Cranial Suture Closure	Pubic Symphysis	Auricular Surface	Fourth Rib	Cranial Suture Closure
AGE RANGE	BELLEVILLE M+F (Saunders et al. 1992)											
19-29		10,3		3,4		0,8		11,1	12,7	3,9	11,1	5
30-39		-2,7		7,8		11,1		5,1	4,4	9,1	11,1	7,1
40-49		-13		-4,4		-2,5		-6,3	13	4,4	7,1	9,5
50-59		-18,5		-7,6		-9,1		-12,9	20,1	9,1	9,1	12,9
>60		-22,4		-16,6		-15,7		-24,6	22,4	16,6	16,6	24,6
TOT	35		53		27		33					
	BOLOGNA M+F											
19-29	36	2,8	47	6,91	20	-0,5	44	15,1	4,6	7,2	4	15,1
30-39	32	3,1	37	-0,04	11	-3,9	36	6	5,6	3,1	7,7	6,9
40-49	22	1,8	28	-1,38	6	2	27	0,7	6	5,9	6	4,6
50-59	29	-9,6	36	-7,32	12	-14,5	36	-7	10,6	8,5	16	7,6
>60	70	-19,7	80	-19	14	-20,1	86	-24	19,7	19	20,1	24
TOT	189		228		63		229					
	COIMBRA M+F											
18-29	30	3,3	35	8,8	9	-2,1	39	13,1	4,1	8,9	2,1	13,1
30-39	37	2,3	44	1,3	8	2,4	44	6,8	4,3	3,4	6,4	7,6
40-49	33	-1,1	38	-2	13	1,1	38	0,7	3,4	5,5	8,2	4,9
50-59	40	-6,4	42	-5,9	9	0,4	44	-7,1	8,6	6,8	4,9	7,6
>60	71	-18,7	70	-18,8	25	-14,8	76	-24,4	18,7	18,8	14,9	24,4
TOT	211		229		64		241					

Table 4.5. indices of bias and inaccuracy obteinde by Sauders et al. (1992) compared to the index of this study.

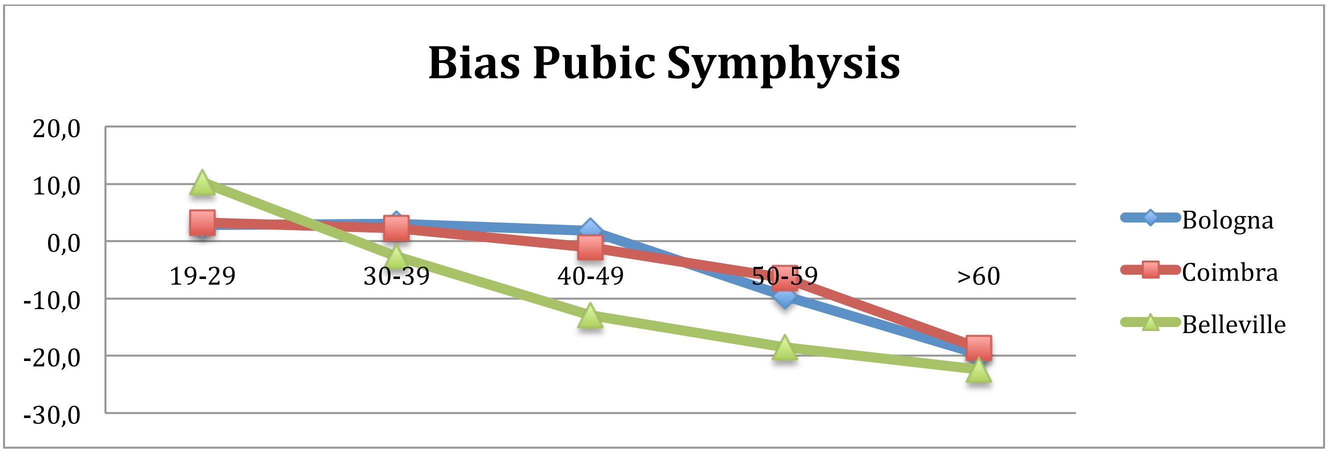
A comparison between the results of bias and inaccuracy of this studuy and the values published by Sauners et al. (graphs 4.21-4.28) shows different results with few exceptions.

In the sample of Belleville the values of bias overestimate the know age by 10 years in the 19-29 age range with the pubic symphysis and sutures closure methods. In the same age range the overestimation considerably decrease in the auricular surface and 4th rib methods. In the sample of Bologna and Coimbra the bias overestimation is almost the opposite, the value of auricular surface method is higher than the value of pubic symphysis method. The bias value of the cranial suture closure method is higher than the Belleville sample.

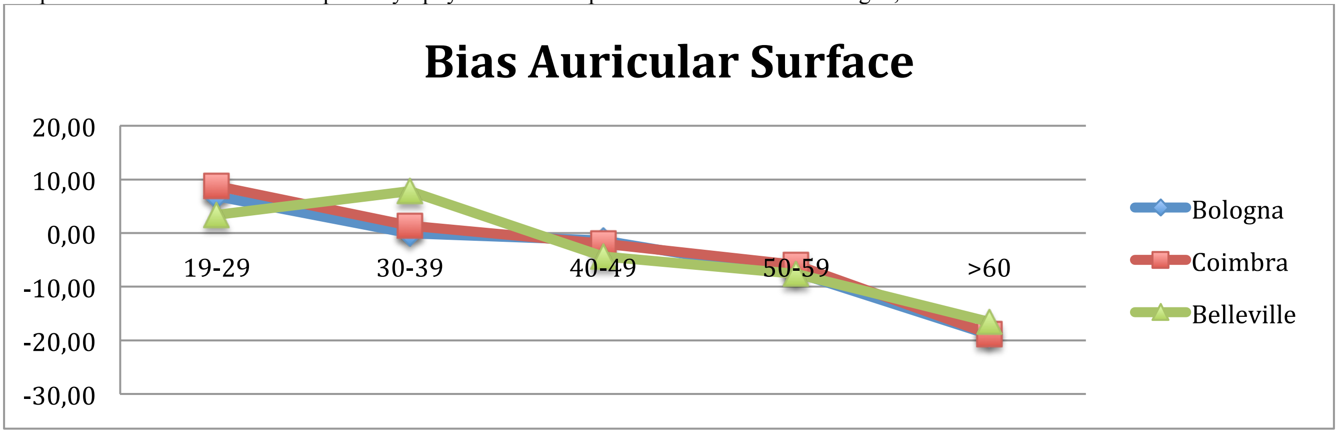
The underestimation starts at 40 years in all the methods except for the pubic symphysis where starts at 30 years.

The bias values correspond in the auricular surface method in the 50-59 age range.

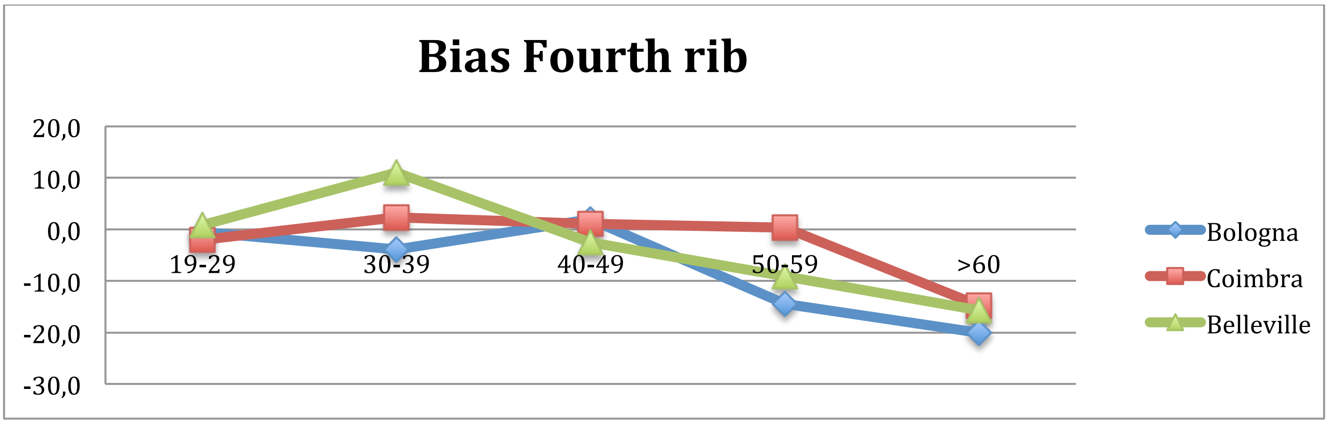
For what concern the inaccuracy the results of three samples tend to merge in the range 30-39 for the pubic symphysis and cranial suture closure methods while with auricular surface and 4th rib methods tend to merge in the range 40-49.



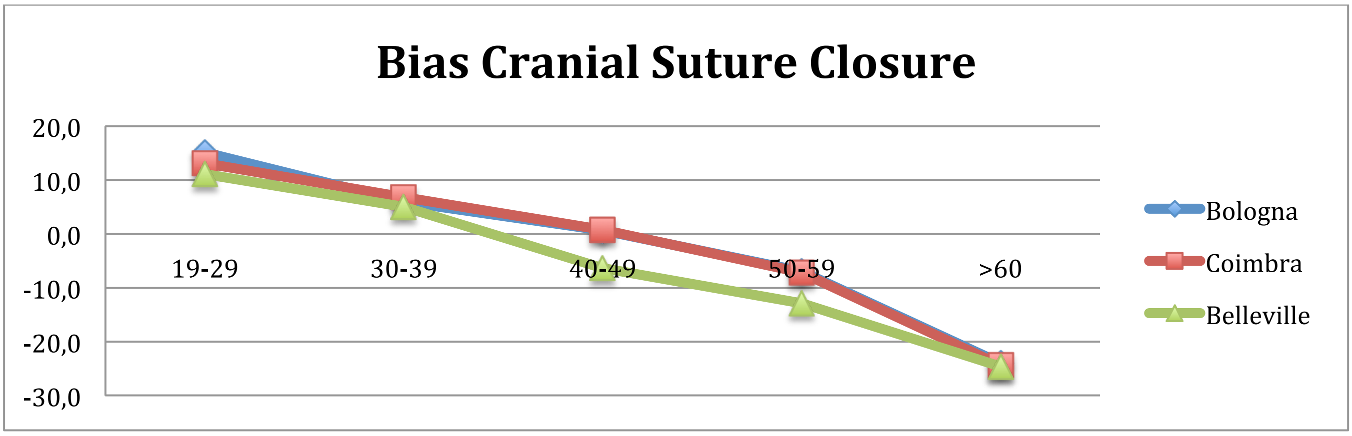
Graph 4.21. values of bias of the pubic symphysis in the sample of the collection of Bologna, Coimbra and Belleville



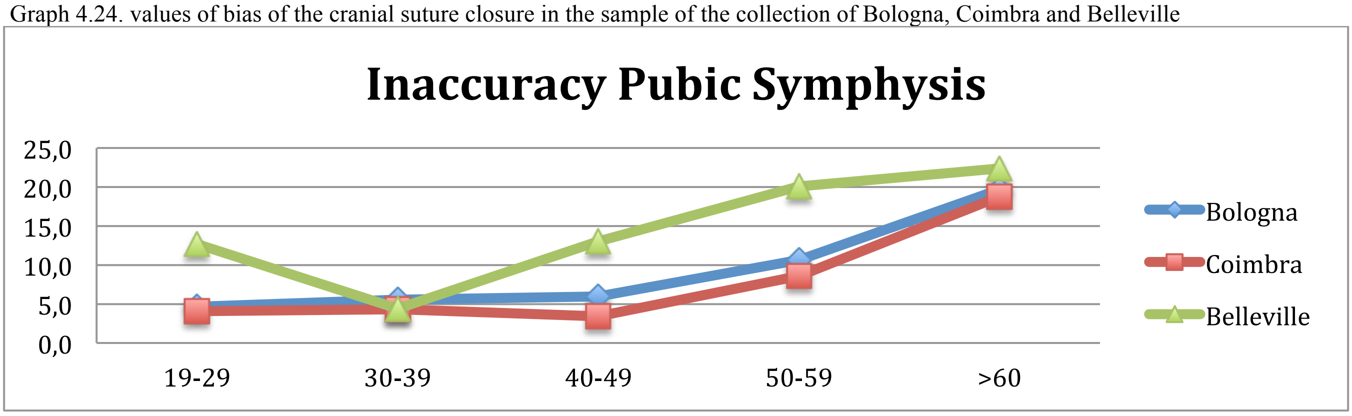
Graph 4.22. values of bias of the auricular surface in the sample of the collection of Bologna, Coimbra and Belleville



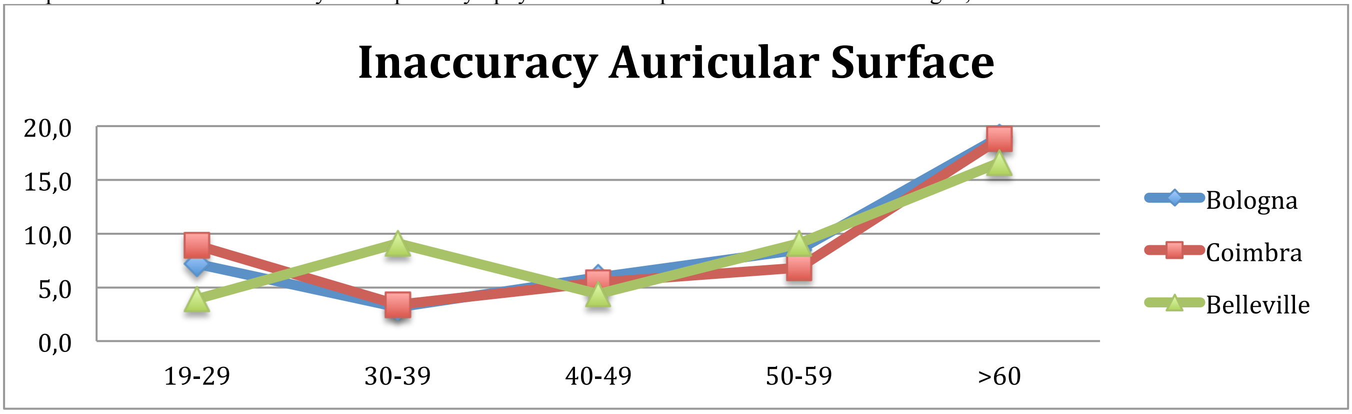
Graph 4.23. values of bias of the fourth rib in the sample of the collection of Bologna, Coimbra and Belleville



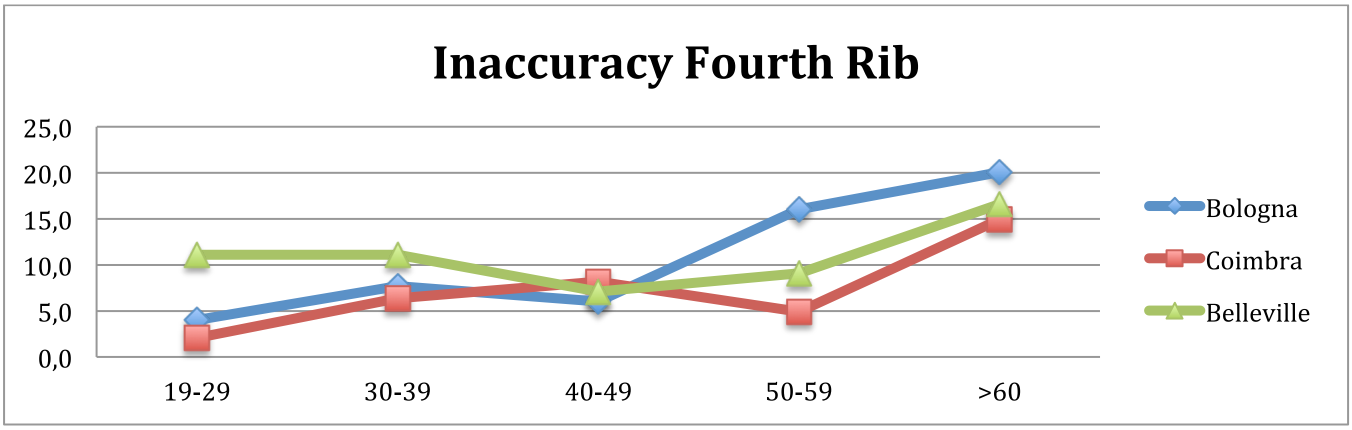
Graph 4.24. values of bias of the cranial suture closure in the sample of the collection of Bologna, Coimbra and Belleville



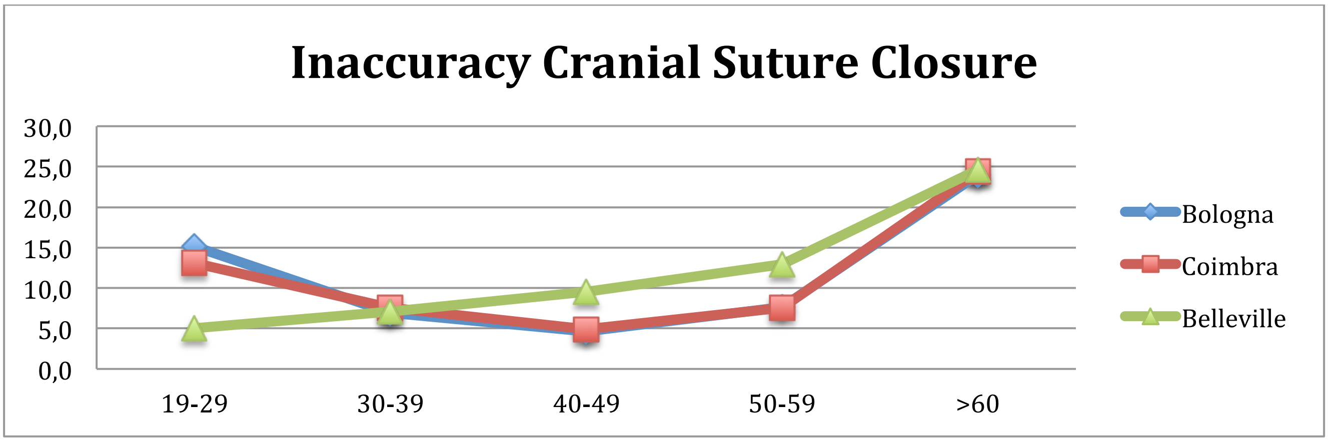
Graph 4.25. values of inaccuracy of the pubic symphysis in the sample of the collection of Bologna, Coimbra and Belleville



Graph 4.26. values of inaccuracy of the auricular surface in the sample of the collection of Bologna, Coimbra and Belleville



Graph 4.27. values of inaccuracy of the fourth rib in the sample of the collection of Bologna, Coimbra and Belleville



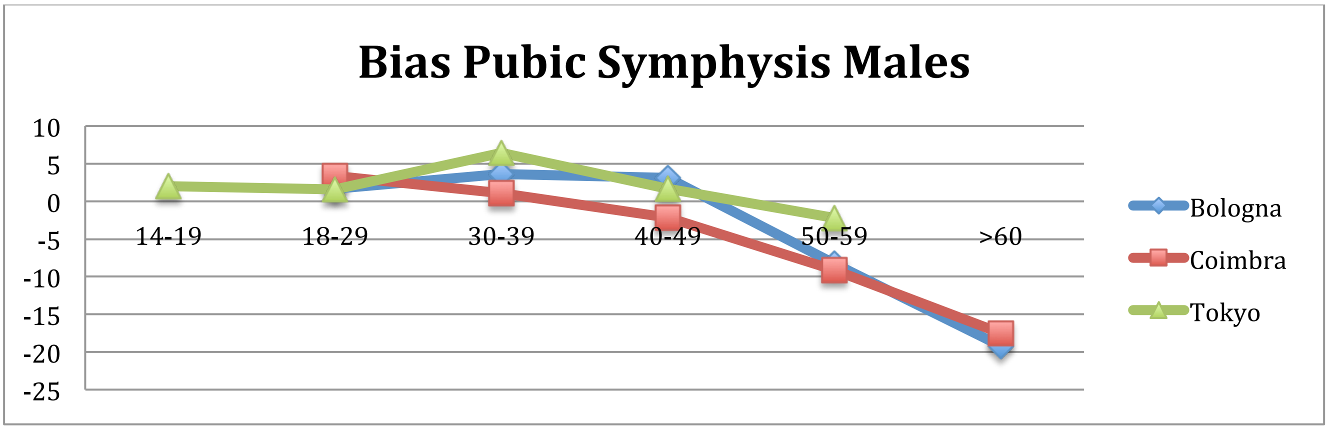
Graph 4.28. values of inaccuracy of the cranial suture closure in the sample of the collection of Bologna, Coimbra and Belleville

- the collection of the Department of Anatomy, (University of Tokyo; Sakaue, 2006)

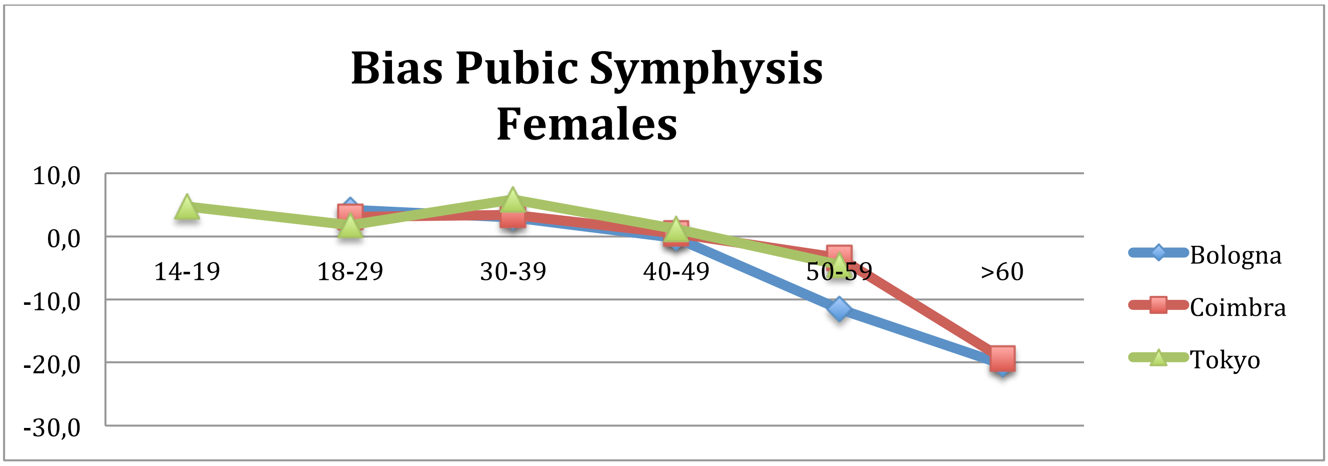
Sakaue (2005) tested for the first time the Suchey-Brooks method in a japanese population of 416 individuals (the collection of the Department of Anatomy, University of Tokyo) (table 4.5).
A comparison between the results of that study with the results of this one shows that the values of bias and inaccuracy follow the same trend. The values of bias tend to correspond in the 18-29 age range in the male samples and in the 40-49 age range in the female samples.

	MALE				FEMALE		
	BIAS		INACCURACY		BIAS		INACCURACY
	N.	Pubic symphysis			N.	Pubic symphysis	
AGE RANGE	TOKYO (Sakaue, 2005)						
14-19	-	4,7	4,7	14-19	-	2	2,4
18-29	-	1,8	3,2	18-29	-	1,6	3,3
30-39	-	5,9	6,5	30-39	-	6,4	7,9
40-49	-	1,2	4,9	40-49	-	1,7	5,8
50-59	-	-4,5	6,7	50-59	-	-2,2	8
	BOLOGNA						
18-29	20	1,66	4,1	18-29	17	4,2	5,2
31-39	16	3,65	6,2	30-39	18	3,1	5,3
40-49	13	3,1	6,6	40-49	9	-0,2	5,2
50-59	1	-8,25	9,8	50-59	9	-11,5	11,5
60+	43	-19,2	19,1	60+	28	-20,3	20,3
	COIMBRA						
20-29	16	3,4	4,2	19-29	14	3,1	4
30-39	19	1,1	3,7	30-39	18	3,5	5
40-49	20	-2,1	4,1	40-49	13	0,4	2,3
50-59	21	-9,2	11,1	50-59	19	-3,4	5,8
60+	26	-17,5	17,7	60+	45	-19,4	19,4

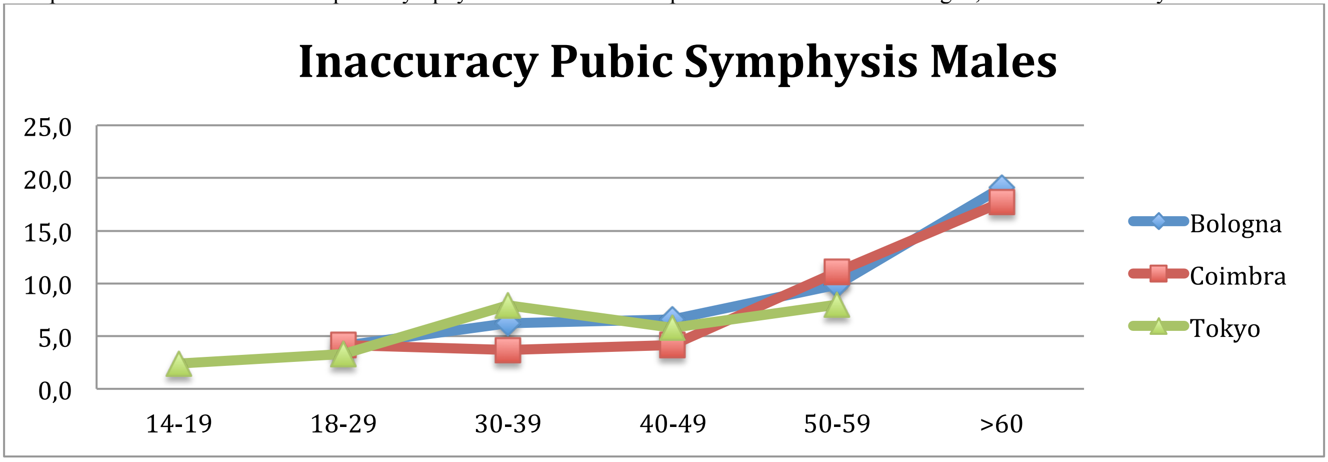
Table 4.5. indices of bias and inaccuracy obtained by Sakaue (2006) compared to the index of this study.



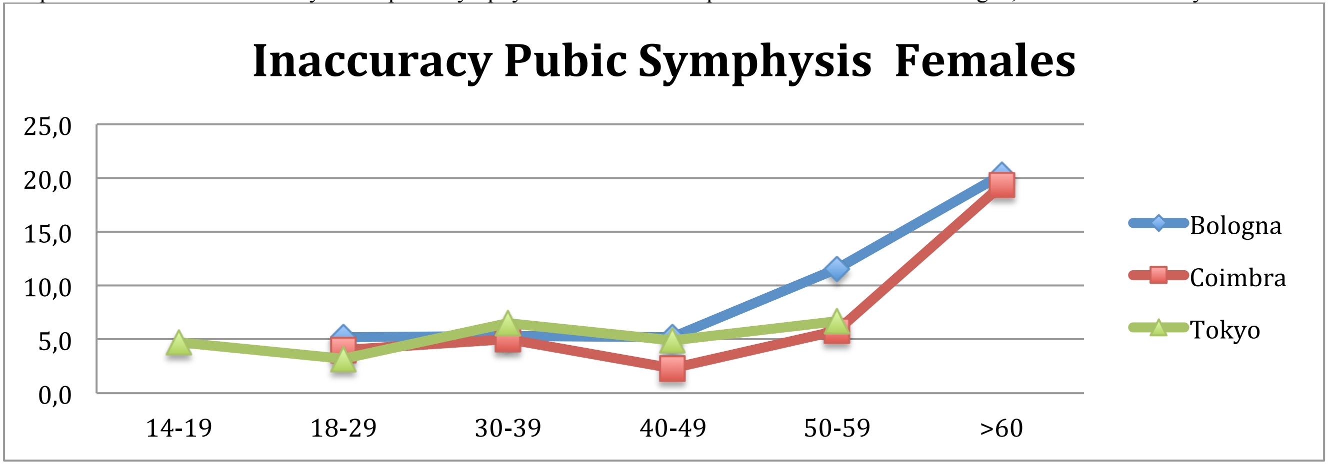
Graph 4.29. values of bias of the pubic symphysis in the male sample of the collection of Bologna, Coimbra and Tokyo



Graph 4.30. values of bias of the pubic symphysis in the female sample of the collection of Bologna, Coimbra and Tokyo



Graph 4.31. values of inaccuracy of the pubic symphysis in the male sample of the collection of Bologna, Coimbra and Tokyo



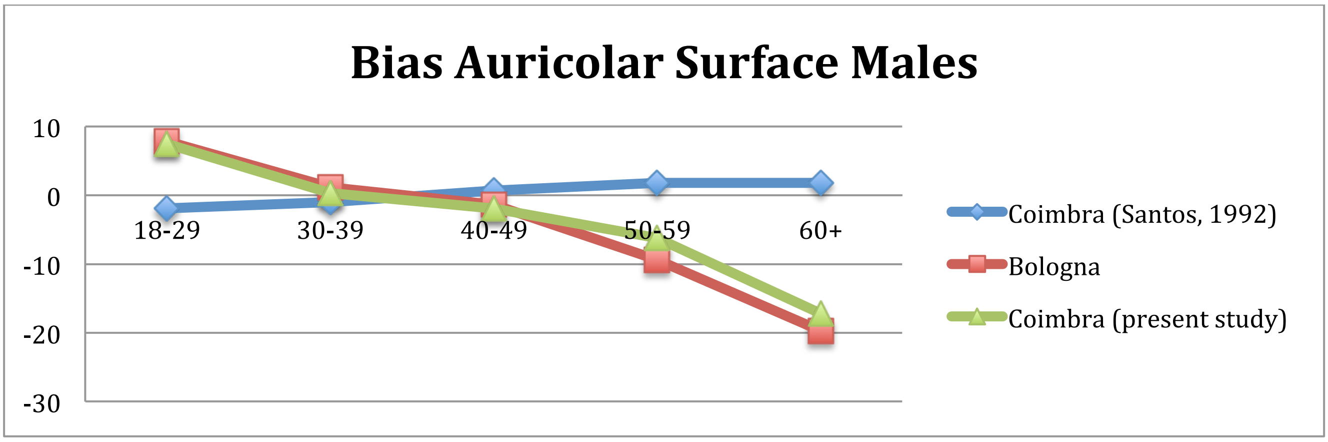
Graph 4.32. values of inaccuracy of the pubic symphysis in the female sample of the collection of Bologna, Coimbra and Tokyo

- the collection of Coimbra (Santos et al, 1996)

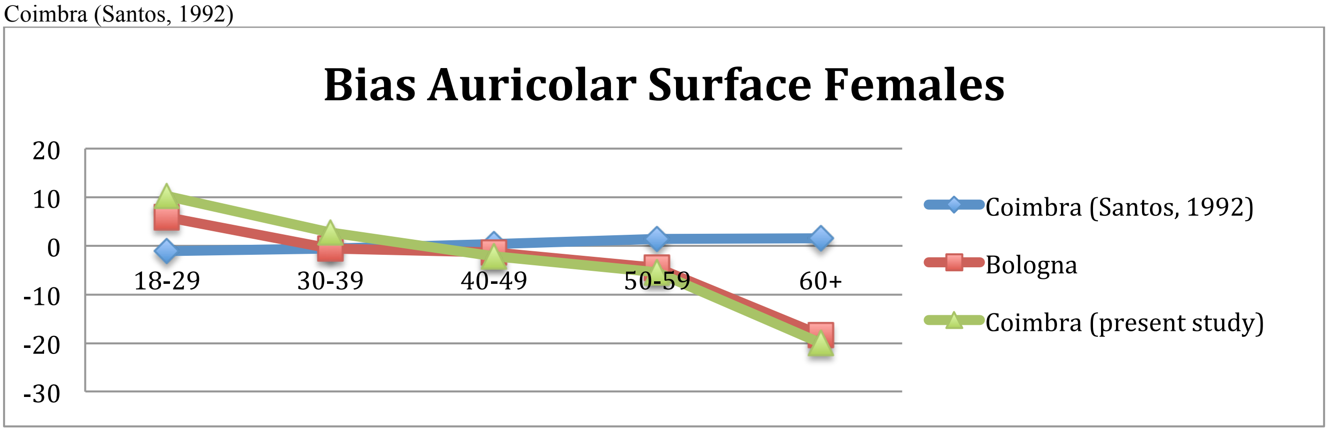
Santos (1992) tested the auricular surface method on a portuguese population of 215 individuals selected from the “Colecção Esqueletos Identificados” of Coimbra.
In this case the method was tested on the Coimbra Collection by two different observers.
In questo caso il metodo è stato testato sulla collezione di Coimbra da due osservatori diversi.
In table 4.6 the results of the present study are compared with the ones of Santos’ study and we can see that the indices find by Santos are very different
both from those of Bologna than from those obtained in this study from the Coimbra sample. The bias values in the present study indicate that the method overestimates individuals between 18 and 39 years (except females of Bologna where the overestimation is between 18 and 29 years) and the underestimation begins from 40 years. In the study of Santos (1992), instead, in the 18-39 age range of individuals the age is underestimated and it begins to be overestimated after age 40.
The inaccuracy values range of this study goes from 1.9 to 20 years while those of Santos are all less than 1.9.

	MALE				FEMALE		
		BIAS	INACCURACY			BIAS	INACCURACY
	N.	Auricular surface			N.	Auricular surface	
	COIMBRA (Santos, 1992)						
18-29	16	-1,9	1,9	18-29	12	-1,15	1,15
30-39	17	-0,95	1,45	30-39	16	-0,5	1,15
40-49	20	0,7	1,2	40-49	18	0,3	1,45
50-59	18	1,8	1,9	50-59	20	1,4	1,6
60+	34	1,8	1,9	60+	44	1,6	1,6
	BOLOGNA						
18-29	25	7,82	7,9	18-29	23	5,9	6,1
30-39	19	1,13	4,6	30-39	21	-0,6	1,9
40-49	16	-1,4	6,8	40-49	12	-1,3	4,6
50-59	22	-9,4	10,6	50-59	14	-4,5	5,8
60+	41	-19,7	19,3	60+	40	-18,4	18,3
	COIMBRA (present study)						
18-29	21	7,5	7,8	18-29	16	10,3	10,3
30-39	24	0,3	2,6	30-39	19	2,7	4,5
40-49	23	-1,9	5	40-49	15	-2,1	6,2
50-59	22	-6,2	6,5	50-59	21	-5,4	6,8
60+	23	-17,2	17,2	60+	46	-20	20

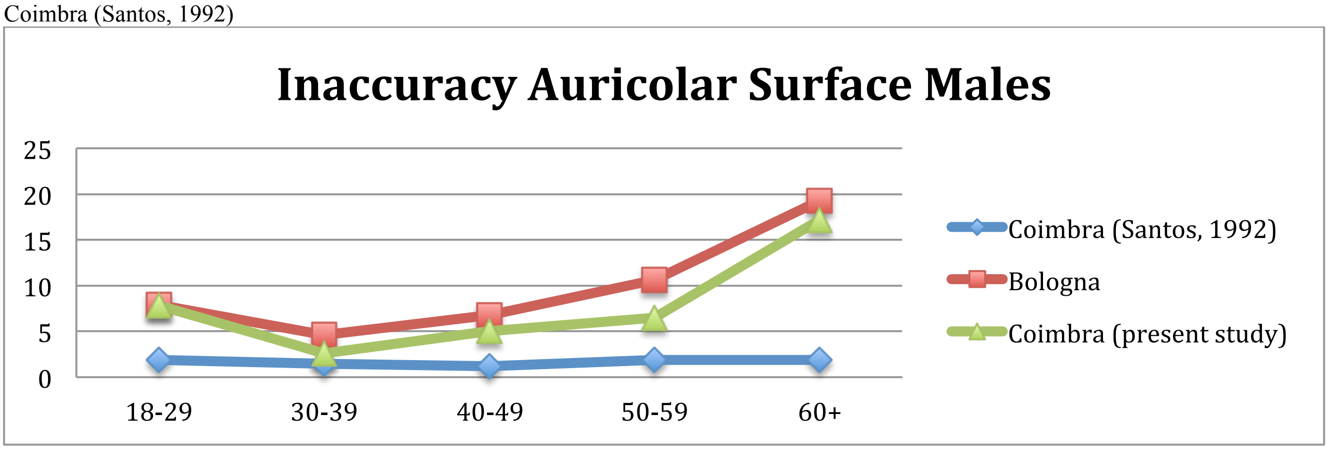
Table 4.6 indices of bias and inaccuracy obtained by Santos (1992) compared to the index of this study.



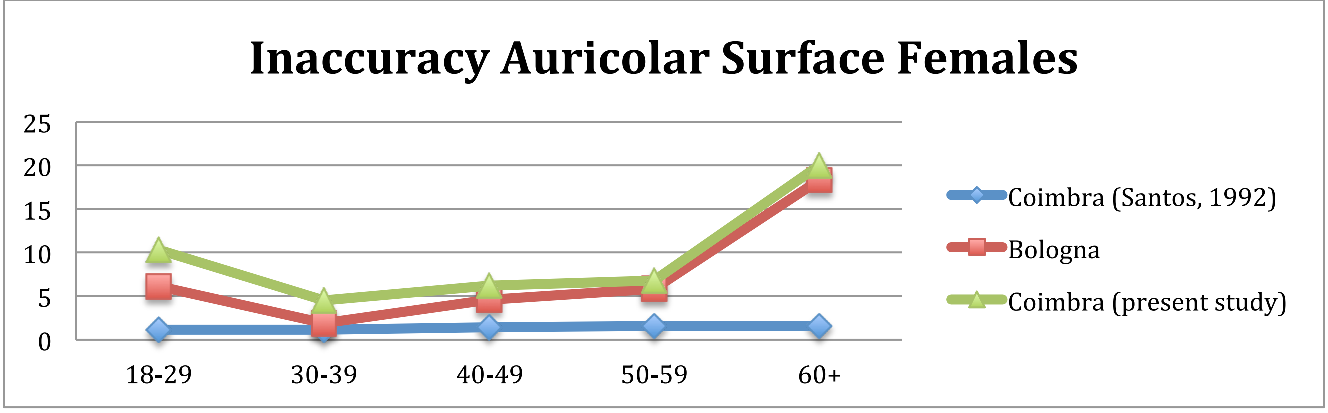
Graph 4.36. values of bias of the auricular surface in the male sample of the collection of Bologna, Coimbra (present study) and Coimbra (Santos, 1992)



Graph 4.37 values of bias of the auricular surface in the female sample of the collection of Bologna, Coimbra (present study) and Coimbra (Santos, 1992)



Graph 4.38 values of inaccuracy of the auricular surface in the male sample of the collection of Bologna, Coimbra (present study) and Coimbra (Santos, 1992)



Graph 4.39 values of inaccuracy of the auricular surface in the female sample of the collection of Bologna, Coimbra (present study) and Coimbra (Santos, 1992)

- the collection of Sassari and Coimbra (Belcastro et al, 2008)

Belcastro (2008) studied the degree of sacral vertebral body fusion in adult in two european modern skeletal collection: Sassari and Coimbra.

I make a comparison beetween the results of this study and the results obeteined by Belcastro et al. (2008).

In my study I considered only 3 age class, “young adults” (YA, 20-34 years), “middle adults” (MA, 35-49 years) and “old adults” (OA, >50 years) (c.f. Buikstra and Ubelaker 1994) considering the smaller size of my sample.

In the three collection (Bologna, Sassari e Coimbra) the degree 3 is the most frequent: in the sample of Bologna was present in all site in all age class considered in male and female group. The complete fusion degree was more frequent in sites S2-3, S3-4, S4-5.

The degrees 1-2 were more frequent in individuals over 35 years in the collection of Sassari and Coimbra wile in the collection of Bologna were more frequent in YA.

The degree 0 was more frequent in individual under 25 years old in the collection of Sassari and Coimbra and in the collection of Bologna were more frequent in YA.

In this study were identified 13 different pattern of sacral vertebral body fusion on 159 indivuduals studied, in the study published by Belcastro et al. (2008) were found 62 different pattern on 823 indivuduals studied.

In both study the most frequent pattern was “a”, x333. The sample of Bologna show a bigger percentage of 90% against the 86% of the other study. In this type in both studies the most frequent pattern was 3333 and there was an increase of the presence with anging.

The types “b” and “c” were very less frequent in this sample to do a comparison. Only the type “d” was present with 21%, and in the collection of Sassari and Coimbra the presence of this type decrease with age with the exception of the female of Coibra.

McKern and Stewart (1957) reported that ‘‘by 23 years ossification is complete, except often between the S_{1-2} centra, where a gap may persist until the 32nd year’’ in their exclusively male sample. But their results can been influenced by the little size of the sample (34 individuals above 33 years). In our sample the site S1-2 has the highest variability of fusion degree.

For the score in both studies the values increase with years and lower part of the sacrum presents less variability and the maximun value of score were in the lower sacrum.

The minimum score was 2,18 in the male sample while in the compared study was 1 in the same sample.

4.2) Comparison of the results of the Phenice method and the results obteined in other study

In our study the method of Phenice confirms the known sex in 87% of the all sample, while Phenice (1969) estimates a percentage of reliability of about 96%. Ubelaker and Volk (2001) applied this method on 198 individuals and confirmed the known sex in 88,4 % of the sample (table 4.8).

Ubelaker (2001) noticed that by using the combined method (estimate sex such as the morphology of the sciatic notch, subpubic angle, auricular area, preauricular sulcus, acetabulum, and dorsal pubic pitting, as well as general pubic morphology) the number of wrong diagnosis decresed from 23 to 7.

Lovell (1989) tested the method on 36 individuals (13 males and 23 females), randomly selected from a larger series of pubic bones obtained from bodies for medical school of known sex. In his study estimated a reliability of the method of about 83%.

The % of the subsample are represented in table 4.8, the higher values are in the sample of Bologna.

MacLaughling and Bruce (1990) tested the method on three European identified collections (table 4.8) and obtained a relaiability between 46 and 93%.

Moreover, he identified the subpubic concavity as the single most reliable indicator of sex, and the same result was obteined in the sample of Bologna and in the male sample of Coimbra (table 3.16).

	MALE		FEMALE	
	N.	%	N.	%
Italy (present study)	96	85	82	95
Portugal (present study)	90	87	95	83
Terry Collection (Ubelaker and Volk, 2001)	99	79,8	99	97
Lovell. 1989*	13	82	23	83
English (MacLaughling and Bruce, 1990)	52	71,7	33	93,7
Dutch (MacLaughling and Bruce, 1990)	72	68	64	68,7
Scottish (MacLaughling and Bruce, 1990)	28	46,4	24	70,8

Table 4.8. Comparison of data of the present study with the data of MacLaughling e Bruce (1990), Ubelaker and Volk (2001) and Lovell (1989). *unidentified origin

CHAPTER 5: Discussion

5.1) Age estimation methods

– age estimation based on the pelvis: Suchey –Brooks (1990) e Lovejoy (1985)

The method of Suchey –Brooks was developed on a bigger sample then the other methods tested in this study. The size of the sample was very important to observe the inter- and intra- population variability of the senescence process.

In this study, the method of the pubic symphysis is the most reliable for estimating age in the sample of Bologna and Coimbra, with a positive match of 91%; follow by the fourth rib with 50% and by the auricular surface with 39% (table 3.2).

In the European samples of Bologna and Coimbra, as well as in the sample of Sassari (Hens, 2008) the known age is underestimated after age 40, and in the present study the Suchey-Brooks method becomes less reliable after 50 years. Similar results were obtained also in other collections (Suchey et al., 1986; Baccino et al., 1991; Saunders 1992; Lovejoy et al., 1995; Schmitt, 2004; Rivera-Sandoval, 2014).

The comparison with other studies shows a variable response of the populations to the application of the method in the range between 40 and 50 years, where it was observed both overestimation and underestimation depending on the studied population, unlike the confirmed greater reliability under 30 years and worst reliability after 60 years.

This method demonstrates a good reliability probably due to the large size of the sample on which it was developed and the relative ease in identifying the various phases.

The different geographical origin seems not to affect too much the reliability of the method. A comparison of our data, obtained from an European sample, and previous studies showed that the method of the pubic symphysis should be applied with caution on the Asian population, but instead has a good reliability on European pulations of Bologna and Coimbra. The major discrepancies were found by comparison with the Thai population (Schmitt, 2004) but we must consider that the sample of Chiang Mai is also the smallest (N. 66).

In all the examined populations the index of bias and inaccuracy increase with increasing age, particularly after 60 years. This was expected since the method includes the older individuals in a single group (>60 years), however bias and inaccuracy increase gradually with ageing, showing that as soon as the pubic symphysis loses its juvenile features, the age range associated to a certain morphology goes wider and therefore less accurate.

This data confirms that more advanced stages of the ageing process are influenced by a complex interaction between exogenous and endogenous factors that act in different ways and times depending on the population. The ageing process may also be influenced by culture, and environment, which determinate the rate and degree of changes in individuals life history. The studied populations appears to age slower than the reference sample as far as this age marker is concerned, as age is consistently underestimated in the 40 years and older subsample.

Considering the differences between sex in the reported studies, the pubic symphysis method is the most reliable in the female sample of Coimbra and Bologna, followed by the sample of Tokyo

(Sakaue, 2006), Sassari (Hens, Belcastro, 2008) and Chiang Mai (Schmitt, 2004). On the overall sample (female+male) the method is more reliable in the European sample than the Colombian (Rivera-Sandoval, 2014) and Canadian samples (Saunders et al., 1992).

The method of auricular surface was developed using a sample of 250 individuals from Libben collection samples (dating from late first millennium and of modern African Americans and European Americans), and a sample of roughly 500 modern and contemporary Americans from the Hamann-Todd collection and 14 forensic cases (Cuyahoga Country Coroner's Office). The values obtained from the sample of Bologna are similar to those obtained from previous studies
(Lovejoy et a., 1985; Murray and Murray, 1991; Saunders et al., 1992; Bedford et al., 1993; Schmitt, 2004).
Moreover, this method is more reliable from 30 to 49 years but if compared to the pubic symphysis method, it shows an overestimation of 10 years for individuals between 18-29 years. The underestimation values for this method start over 60 years.

The method underestimates the age of older individuals and overestimate the younger ones, therefore this method is less reliable if used alone to estimate age.
The changes to the auricular surface with age are more complex to identify than those occurring in the pubic symphysis. This makes the former method much more difficult to apply then the latter, and the experience of the observer is an important factor.
This problem was also been expressed by the authors (Lovejoy, 1985) who believed that "is more difficult to interpret than those used in pubic symphyseal ageing"
The method proposed by Lovejoy et al. (1985) had already been applied to the collection of Coimbra (Santos, 1992). The results were not encouraging, only the 17,7% of the sample had been properly classified. In the study published by Santos (1992), as well as in the present study, it has been studied only a part of the collection, selected randomly; in this study are more represented individuals under 60 years, the age group in which methods give better results (see table 2.3). These differences in the composition of the sample may in part explain the different results, but that is probably due to the inter-observer error. Moreover Santos (1992) has emphasized how an ambiguous definition of the characteristics in the original works, especially as regards the morphology of the auricular surface, has weighed on the estimation of age.
The articular surface joints and pubic symphysis are different joint with different motility. The joint between the sacra and the pelvis is subject to movements of slipping also is also subject to the weight of the spine and it can present osteoarthritis of the two articular heads. This pathology can lead to a premature ageing of the articular surface. Such factors may cause more difficulty for reading and classifying of the surface.
In the sample studied the overestimation in the range 18-29 years could confirm the hypothesis of a possible premature ageing of the joint due to the functional stress.

It is important to remember that all methods gives a broad age range, (5 - 10, up to 50 years, chapter 2: 2.3), consequently very positive results, as in the case of the pubic symphysis, should be considered in the light of this premise.

In the study has been verified that in case two emi pelvis are found individually, the risk of attributing them to two different developmental phases and refer them as two different individuals do not seems significant. The samples do not show any particular differences with regard to the laterality applying the methods of Suchey-Brooks and Lovejoy (Table 6).
This could be related to the fact that in the studied population the joints studied are subject to mechanical stress evenly in the left and right side, instead of what may happen in other joints such as those of the upper limbs (shoulder, elbow and wrist) and lower limbs (hip, knee and ankle).

- variation of the degree of sacral vertebral body fusion (SVF) (Belcastro et al. 2008)

The variability of SVF generally decrease with age in the collection of Bologna and in both sexes: the frequencies of the degrees 0, 1, 2 decrease with age, while the frequency of complete fusion (degree 3) increases with age. The degree 3 is the most frequent in all site of the sacrum: the type ‘‘a’’ patterns (x333) are absolutely the most frequent and the lower score presents higher values than the upper and total scores, in agreement with the caudo-cranial direction of sacral vertebral body fusion (Rouvìere, 1940; McKern and Stewart, 1957; Johnston, 1961).
The application of the SVF method for the adult age estimation in this study shows that most adult sacra are completely fused (70% of the examined sacra), the incompletely fused sacra can be attributed to young adult individuals (20–34 years).
The complete sacral vertebral fusion indicates usually only adulthood and does allows to distinguish among young, middle and old adults.

- obliteration of the cranial sutures (Meindl e Lovejoy, 1985)

Some studies point out that the degree of obliteration of these suture could be useful in the estimation of the age at death whereas some other studies considered it unreliable.
In this study the method of cranial sutures closure is the one with the more limited range of reliability compared to the other methods tested; in our European sample it is more reliable between 40-49 years.
The values of bias and inaccuracy show a similar trend in all samples, and gives values higher than the values of the pubic symphysis and the auricular surface. There aren’t major differences between the sexes, as reported by the authors (Meindl and Lovejoy, 1985).
The study does not confirm the conclusions of the authors that consider the lateral anterior system more reliable in the age estimation than the vault system.
Such data may indicate that also the obliteration of the sutures may be influenced by interaction between exogenous and endogenous factors that act in different ways and times depending on the examined population.

Moreover, the method has been developed on a sample of 236 skulls; such sample could have been not representative of human variability and therefore its reliability would be much lower on different populations.

For the present study, it has been considered a sample of 473 skulls, probably with a larger variability.

From a comparison with the study of Saunders et al. (1992), instead of the results obtained by the authors, it shows that the method provides very similar results in all the 3 populations compared and then the sutures may not be affected by exogenous and endogenous influences.

- modifications of the sternal end of the fourth rib (Iscan et al., 1985, 1985, 1986)

The method of the fourth rib was developed on a contemporary population of North Americans with European ancestry. The sample of the author is smaller compared to the one considered in this study and the sample on which it was possible to use the method of the fouth rib is the smallest in the study. The results on our European sample show a 50% of positive match.

The comparison with the results of Sander et al. (1992) show different trends, only the underestimation over 60 years is common in the three populations. This method is the one with more inter population differences under 60 years, demonstrating the lower reliability in the tested populations.

For this method the sample size seems to have a particular importance, despite the size of the sample considered in this study is superior to that of the authors, the results obtained are not conclusive (table 3.2).

However it must be noticed how in bioarchaeology the application of this method is severely limited because of the fragility of the district which makes its availability very rare in individuals from archaeological contexts.

To improve these data it would be necessary to have a larger sample for each age group.

- consideration on the simultaneous use of different methods for the age estimation

Some authors (Murray and Murray, 1991 Santos, 1992; Baccino et al., 1999) point out that considering the different indicators of age in the skeleton is much more effective than working with a single method, a situation that can be affected by the experience and training of the researcher. Not everyone came to this conclusion, Saunders et al. (1992) argues that the use of different methods for the estimation of age do not provide a better result compared to a single method.

This study confirms the usefulness of using multiple methods simultaneously: considering the overall reliability of the different methods each one is more reliable in a decade, allowing a reliable age estimation from 18 to 50 years across the sample (pubic symphysis - range 18-29, auricular surface - range 30-39 and cranial sutures closure - range 40-49; table 3.5).

It also confirms the need to improve the methods of age estimation of the older sample, over 60, which is find systematically underestimated in all methods tested.

One of the most significant problems encountered in age estimation of the adults, that is also pointed out in this work, concern people with more than 60 years.

5.2) Sex determination

- The index of sexualization of Acsadi & Nemeskeri (1970)

The results obtained by the index of sexualization allows to estimate a different degree of sexual dimorphism in the populations examined. Dimorphism is more evident in the population of Coimbra where the index values are closer to +2 and -2. However, the method allows for a correct sex attribution in 99% of the total sample.

The coefficient of the skull has confirmed the known sex in 468 on 484 skulls examinated. This result is important for biarcheological applications because it is a district that is preserved more easily than the pelvis due to his resistance to the taphonomic processes .Also from a "cultural" point view, the skull is subjected to aimed preservation measures in the case of secondary depositions for the specific interest related to this district in terms of cultural and ritual values.
The index of sexualization of the pelvis is even more reliable results than those of the skull, but this specific characteristics of this district was already known (Ferenbach et al. 1980; Krogmanand and Iscan 1986; MacLaughlin and Bruce 1990; Walrath et al., 2004; Gonzalez et al., 2007).

In some individuals of the sample of Bologna the single district, skull or pelvis, do not provide a correct diagnosis but the average of the two coefficients always confirms the known sex in the whole sample (table 3.14). Considering this data and the high number of hyperfeminine and hypermasculine indexes of the pelvis (graph. 3.26) we can point out that in this population, the pelvis has a higher value than the skull.
In the sample of Coimbra the average does not confirm the known sex only in two cases (table 3.14) when the pelvis gave the incorrect diagnosis and the skull the correct one; this represent an exception to the common observation that the pelvis is the more discriminant body part.
Whereas the skull and pelvis provide a discordant diagnosis (19 individuals) the average coefficient confirms the known sex in 89% of cases (17/19) but we must consider both skeletal districts for a correct diagnosis. Also in this case consider more district to determinate the diagnosis it proves to be better.
Masset (1987) reports an error of 20% in sex determination from the skull, Meindl et al. (1985) suggests that it is due to the different expression of the sexual dimorphism of skull and pelvis.
These authors showed a low correlation between sexual dimorphism of skull and pelvis (Walrath et al., 2004). In the present study the error of the skull is 3%.
According to the results of the index of sexualization the individuals of Coimbra show sexual features more marked than the individual of Bologna: 64% of female individuals is in the hyperfeminine range (from -2 to -1) and 68% of male individuals is in the hypermasculine range (from 1 to 2) (graph 3.27).
The problem of different expression of sexual dimorphism is constant because it depends on population (Meindl et al., 1985; MacLaughlin and Bruce, 1986, 1990; VanVark and Schaafoma 1992).

- the Phenice method (1969)

The Phenice method gave good results, even though the value of 87% confirming the known sex of the present study which is similar to that obtained by other studies (Lovell, 1989; MacLaughlin and Bruce, 1990; Ubelaker, 2001), only Suttherland and Suchey (1987) obtained the 96%, the same percentage determined by the author. This percentage was obtained by using the same system of classification of the ambiguous morphological sex indicators of the author: “When there is some ambiguity concerning one, or rarely two of the criteria, there is almost always one of the criteria which is obviously indicative of male or female.” (Phenice, 1969 p.300) (McFadden and Oxenham, 2016).

In the sample of Bologna the component with the highest percentage of reliability is the subpubic concavity as found by MacLaughlin and Bruce (1990) while in the sample of Coimbra is the ischiopubic ramus for the male sample and the ventral arc for the female sample.

Sutherland and Suchey (1987) concluded that ventral arc is the most reliable component but in the present study this conclusion is confirmed only in the female sample of Coimbra (table 3.16).

Considering the comparison of the study the district with the best reliability is not the same in all sample, this difference is probaby a consequence of the inter-population variability.

Moreover the comparison show the best reliability in the female sample in all studies considered (Lowel, 1989; MacLaughling and Bruce, 1990; Ubelaker and Volk, 2001).

The application of the Phenice method to pubic bones of both sides do not show bilateral asymmetry in the expression of dimorphic features.

In the case of archeological samples in which is preserved only one side, the sex diagnosis is reliable applying this method.

Our results show that the combination of the two tested methods (the Phenice method and index of sexualization) give more reliable results than the use of a single method.

In these populations sex determination appears as a relatively more manageable task compared to the problem of age estimation.

CHAPTER 6: Conclusions

The purpose of this study was to test the reliability of the methods commonly used in bioarchaeology and forensic anthropology to assess the age and sex of human skeletal remains on modern identified osteological collections from Italy (Bologna) and Portugal (Coimbra). These methods are based on the macroscopic observation of morphological features recognized as sex and age skeletal markers. The synchronic and diachronic variability of human populations represents a major problem in the application of these methods on skeletons belonging to different populations from those on which the methods themselves have been developed.

This work aims to extend the number of skeletal collections on which these methods have been tested, adding new and important information on the variability of sexual dimorphism and of the ageing processes among human populations.

Most of the methods for sex and age estimation have been developed on American collections including the skeletons of individuals of different ancestry. For this reason, testing these methods on European collections is particularly important.

The Coimbra collection is one of the largest and better documented European identified skeletal collection, and has been widely studied by bioarchaeologists from all over the world. For the Bologna collection, a detailed check of the personal information regarding each individual from the archives of the Certosa cemetery of Bologna was concluded in Spring 2015. Even though smaller than the Coimbra sample, the Bologna sample is now one of the best documented human skeletal collections.

The methods for age estimation tested in this work are based on the morphological changes of the pubic symphysis, the auricular surface of the ilium, the sternal end of the fourth rib, the synostosis of cranial suture and the variation degree of sacral vertebral body fusion. For sex determination the methods are the index of sexualization and Phenice method.

The results of this study show that the pubic symphysis is the most reliable age marker in the sample of Bologna and Coimbra (positive match of 91%), followed by the fourth rib (50%) and by the auricular surface (39%). The method of cranial sutures closure is the one with the more limited range of reliability compared to the other tested methods.

The index of sexualization confirm the known sex in 99% of the total sample.

The coefficient of the skull alone confirms the known sex in 95% of the individuals.

Phenice method gave good results, confirming the known sex in the 87% of the sample.

The study show that the pelvis is the most reliable district for the age estimation and the sex determination; from the comparisons with other studies is it possible to appreciate that the skull is a strongly dimorphic district in the studied populations, almost as much as the pelvis.

This is very important because the skull is often better preserved than the pelvis in bioarchaeological remains and sometimes it can be all that is left of an individual.

Furthermore there are differences between populations concerning the suture closure system: in fact the study does not confirm the conclusions of the authors considering the lateral anterior system more reliable in the age estimation than the vault system.

This study suggests that the methods that will be developed in the future for the age estimation must be more detailed in the individuals over 60 years specially in the forensic context.

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