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THIRD MOLAR MATURITY INDEX BY MEASUREMENTS OF OPEN APICES IN A LIBYAN SAMPLE OF LIVING SUBJECTS

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Abstract

In most countries, forensic age estimation in living subjects has become increasingly important in the last few years. In addition, as the age of legal majority ranges from 14 to 18 in many countries, and in Libya it is 18 years, radiographic assessment of the degree of third molar development is essential for forensic age estimation of adolescents and young adults. The aim of this paper is to assess the accuracy of the cut-off value of 0.08, by measurements of third molar index (I_{3M}), in determining if a subject is adult or not in Libyan population. Digital panoramic radiographs of 307 healthy subjects (163 girls, 144 boys), aged between 14 and 22, were analysed. The $I_{3M,}$ the age and the sex of the subjects were used as predictive variable for age estimation. Using a cut-off of 0.08, the sensitivity of the test for boys was 90.9% and the specificity 100%. The proportion of correctly classified individuals was 95.1% (95% CI: 91.5–98.7%). The sensitivity for girls was 90.6% and the specificity 100%. The proportion of correctly classified individuals was 94.5% (95% CI: 90.9-98.1%). Estimated post-test probability in boys and girls was 100%. Further analyses, performed using a cut-off of 0.09, do not affect the specificity (100%) while they improve the sensitivity for both boys and girls.

Keywords: Forensic sciences, Age estimation, Third molar index, Libya, Mediterranean, Maturity.

Introduction

In the last few years, age estimation in living individuals has become increasingly common in civil and criminal cases involving young people, irregular immigration and the many issues related to asylum seekers [1-4].

Estimating the age of living subjects often requires an integrative approach that involves anthropology, medicine, forensic dentistry and radiology [2-9]. Human identification and aging using dentition has been well established in the forensic field and several methods based on dental changes have been developed [10-13].

In accordance with the updated recommendations for age estimation in criminal proceedings of the Study Group on Forensic Age Diagnostics, for age estimation, a physical, examination, an X-ray examination of the hand as well as a dental examination should be performed. If the skeletal development of the hand is completed, an additional X-ray examination or CT scan of the clavicles should be carried out. The latest version of these recommendations can be found on the AGFAD (Arbeitsgemeinschaft für Forensische Altersdiagnostik or, in English, Study Group on Forensic Age Diagnostics) website at http://campus.uni-muenster.de/6757.html?&L=1

The most common dental method for age estimation is the Demirjian Staging System (DSS) [14-16]. This method identifies eight developmental stages (A-H) based on the evaluation of the mineralization of a selected number of teeth [17-19]. The score system of Willems et al. [20] is the best adaptation of Demirjian's method and is the recommended method of choice to assess maturity or estimate age if all seven teeth are available [21].

Toward the end of human skeletal growth and development, only a few age-dependent features (third molar development, the closure of the clavicular epiphysis and the ossification of the long bones' epiphyses) can be evaluated by morphological methods [22,23]. Since third molars are still growing at the age of 18, their development has recently been considered the most suitable evidence to assess whether a subject is an adult or not [23,24].

Cameriere et al. [25] developed a new method based on the correlation between the age of the individual and the normalized measures of the open apices and the height of the third molar. A threshold (cut-off) value of 0.08 was identified and used to discriminate between individuals who are or not 18 years of age or older. Furthermore, in many countries, including Libya, the age of 18 represents the legal majority [26].

The age of criminal responsibility in Africa varies by jurisdiction across the continent. It ranges from age 10 in Sierra Leone, Cameroon and Côte d'Ivoire to age 14 in other countries (e.g. Central African Republic, Somalia, Libya, etc.) [27]. In Libya, according to the article 9, Act No. 17 of 1992, regulating the situation of minors, the age of legal majority is 18 years. During the Gaddafi government, the regular age was 18 but 16 years old women could be married as long the parents consented. In addition, in this country pre-marital sex was illegal. After the 2011 revolution, the age of consent limit was removed and, presently, although sex is not allowed outside of marriage, there is no defined age of consent [28,29].

In Libya, the need for reliable and accurate age estimation techniques has never been greater than in the last five years, mainly due to armed conflicts within the country. The lack of a validated method for assessing age in Libyan population is fundamental in criminal proceedings relating to irregular immigration and emigration movements at both national and international levels [28].

To date, only one study that has focused on the dental age estimation in Libyan population has been published [30]. No previous works have used also the Cameriere's method in this human group. In addition, in Africa, no systematic studies have been conducted that could inform us of the maturity parameter variations applicable to its population [31]. Only few studies have been carried out in order to age live individuals who have no record/documentation of their chronological age. Garamendi et al. [32] showed very good results when tried to verify the reliability of combined skeletal and dental methods on a Moroccan origin population. Cavrić et al. [33] analyzed the development of the left mandibular third molar by the third molar maturity index (I_{3M}), established by Cameriere et al. in 2008 [25], and used it for discriminating between minors and adult black Africans from Gaborone, Botswana. Their results indicated that I_{3M} may be a useful alternative method in legal and forensic practice to discriminate individuals of black African origin who are around the legal adult age of 18 years in Botswana. Uys et al. [34] studied a sample of 604 black South African children to test the validity of the method described by Demirjian [14]. In 2009, Phillips and van Wyk Kotze [35] analysed the accuracy of two dental methods, Demirjian et al. [14] and Moorrees et al. [36] in a sample of South-African children aged between 3 and 16 years.

The aim of this paper is to assess the accuracy of the threshold value of 0.08, by measurements of third molar maturity index (I_{3M}), in determining if a subject is 18 years of age or not. For this purpose, a sample of living children and young adults from Tripoli, the capital city where different ethnic groups are represented, was analysed.

Material and methods

Digital panoramic radiographs (OPTs) of 420 healthy living Libyan subjects, aged between 14 and 22, were analysed retrospectively. The sample was randomly selected from the Academic Dental Center in Tripoli (Libya) and collected, for clinical reasons, from January to March 2015. The consent to use them for research and educational purposes was obtained directly from the patients or, when they were under the legal age of 18 years, their relatives are requested to sign agreement with dental institutions without the possibility of personal identification.

Patients' identification number, sex, age (years) were recorded but no further information related with the ethnicity was recollected.

The inclusion criteria required each individual had to be of sound health with known sex and precise age (14 to 22 years) at the time the OPT was obtained. The images had to be of a suitable quality/resolution with minimal distortion.

Exclusion criteria from the study sample are the following: panoramic X-rays images with lost or extracted single rooted teeth, as well as those with fillings, crown restorations, severe caries or other abnormal dental anatomy that may result in inaccurate measurement; agenesis and/or extraction of the third molars; third molars with developmental anomalies (e.g., abnormally short roots, dysmorphology); asymmetric root formation between left and right side; impacted and/or unclear emergence direction. Impacted third molar teeth and third molar mesially, distally and vestibulo-orally angulated were classified as impacted as recommended by Wolf and Haunfelder [37].

The exclusion was performed in other to minimize the reading errors associated with artifact related with the history of the patients and not with the growth of the teeth.

A total of 307 OPTs (163 girls and 144 boys) was finally examined. All the children were from the middle socioeconomic class.

The study was conducted in accordance with the ethical standards laid down by the Declaration of Helsinki (Finland). The World Medical Association (WMA) developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data [38].

Measurements

The selected digital radiographs were saved in JPEG format. In order to adjust grey scale, brightness and contrast, image quality improvement tools in Adobe® Photoshop® CS4 were used. The FDI (Fédération Dentaire Internationale) two-digit system notation was used. According to the previous studies the left side was evaluated [39].

Dental age was assessed according to the method of Cameriere et al. [25]. Since the development of the left and right third molars is strongly correlated, multicollinearity problems in the regression models could be detected [40,41]. Therefore, for standardization, the left side was evaluated. The apical ends of the roots of the left lower third molar of each individual were analysed and the measurements were performed using a computerized image-processing program (ImageJ).

The third molar maturity index, I_{3M}, was defined as follows: if the root development of the third molar is complete, i.e., the apical ends of the roots are completely closed, then $I_{3M}=0$, otherwise I_{3M} is evaluated as the sum of the distances between the inner sides of the two open apices ($A_i, i = 1, . . ., 7$) divided by tooth length ($L_i, i = 1, . . ., 7$). Maturity index I_{3M} is evaluated in a similar way to the ratio A_i to L_i, when I = 6 or 7, as reported for the other two teeth with two roots in Cameriere et al. [25]. Determination of I_{3M} allows the use of a single predicting variable which is achieved by normalizing the values of the width of the apices and height of the teeth [25].

Statistical analysis

Each orthopantomograph was coded with a numerical ID (Identity Document) so as to prevent observer bias, and the observer, therefore, was not aware of the age or sex of the subjects. The age of each individual was reported as communicated by the patient in years.

To assess the reliability of the paired set of measurements, three months after the first evaluation the concordance correlation coefficient was calculated on 100 individuals randomly sampled. All 100 orthopantomograms were then re-evaluated by observers. Randomly selected means that each individual has an equal and independent chance of being selected to belong to the sample of 100 used to test reliability of the method.

Analysis of covariance (ANCOVA) was performed to study the interaction between I_{3M} and the sex.

The correlation between age and third molar index (I_{3M}) was tested with Pearson's correlation coefficient.

The $I_{3M,}$ the age and the sex of the subjects were used as predictive variable for age estimation.

All statistical analyses were performed using the IBM SPSS 22.0 software program (IBM® SPSS® Statistics, Armonk, NY). The significant threshold was set at 5% and 1% as reported on the text.

Based on the results of Cameriere et al. [25] and De Luca et al. [42], the same cut-off value of 0.08 for I_{3M} for both sexes was evaluated, so that an individual is considered to be 18 years of age or older if I_{3M} is lower than 0.08. The third molar index can help to discriminate between individuals who are 18 and over and those under 18 by the post-test probability, or p, of being 18 years of age or older (i.e.: the proportion of individuals with I_{3M} <0.08 who are older than or equal to 18 years). The sensitivity of the test, p1 (i.e.: the proportion of subjects older than or 18 years of age who have I_{3M} <0.08), together with specificity p2 (i.e.: the proportion of individuals younger than 18 who have I_{3M} >0.08) were evaluated.

In addition, in order to improve the discrimination model a cut-off value of 0.09 for I_{3M} for both sexes was tested.

According to Bayes' theorem, post-test probability may be written as:

$$p = \frac{\text{p1p0}}{\text{p1p0} + (1 - \text{p2})(1 - \text{p0})}$$

Where p is post-test probability and p0 is the probability that the subject in question is 18 years old or older, given that he or she is aged between 15 and 22 years, which represent the target population. Probability p0 was calculated as the proportion of Libyans between 18 and 22 years of age who live in the Libya according to demographic data from the World Bank (http://data.worldbank.org/country) and those between 15 and 22 years which was evaluated from data from the same web source. This proportion was considered to be 54.4% for boys and 55.6% for female.

Results

The reliability of the data collection was evaluated calculating the concordance correlation coefficient (ρc) and no statistically significant difference between paired sets of measurements was detected: ρc=1.00.

In this study, carried out on 307 Libyan healthy subjects a minimum of 28 (14 years) and a maximum of 98 (20-22 years) individuals were studied per age and sex (Table 1). Sample scores range from 0.00 to 1.34 depending on the age group as detailed in the Figure 1.

Distribution of real age gradually decreased as I_{3M} increased, in both boys and girls (Fig. 1).

The mean ages for both groups in each I_{3M} class varied between sexes (Table 2) but the differences were not statistically significant (p=0.573). Despite no differences in sexes were detected, in order to be consistent with the previous literature on this topic, the performance of the cut-off value of I_{3M} = 0.08, reported in Cameriere et al. [29] and the validity of I_{3M} on the actual Libyan sample was analyzed for boys and girls separately.

The results of the analysis of the effectiveness of I_{3M} are presented in two 2 x 2 contingency tables (Tables 3a and 4a), which list the numbers of individuals who have $I_{3M} \geq 0.08$ and are younger than 18, those with $I_{3M} \geq 0.08$ who are over 18, those with $I_{3M} < 0.08$ who are under 18, and those with $I_{3M} < 0.08$ who are over 18. Additional two 2 x 2 contingency tables (Tables 3b and 4b) are presented using a cut-off of 0.09.

Table 3a shows the close association between adult age and the positivity of the test using a cut-off of 0.08 ($I_{3M} < 0.08$) in boys. Seventy out of 77 individuals were accurately classified. These results show that the sensitivity of the test for boys (the proportion of individuals being 18 years of age or older whose test was positive) was 90.9% and the specificity of the test (the proportion of individuals younger than 18 years whose test was negative) was 100%. The proportion of correctly classified individuals was 95.1% (95% CI 91.5–98.7%).

Similar to Table 3a, Table 4a shows the close association between adult age and the positivity of the test (i.e., $I_{3M} < 0.08$) in the female group. Of 96 individuals, 87 were accurately classified. These results show that the sensitivity of the test for females was 90.6% and the specificity (the proportion of individuals younger than 18 whose test was negative) was 100%. The proportion of correctly classified individuals was 94.5% (95% CI 90.9–98.1%).

Estimated post-test probability p in boys and girls was 100% being the specificity (p2) 100%.

If a cut-off of 0.09 is applied, it does not affect the specificity that remains 100%. The 0.09 cut-off improves the sensitivity both for boys (96.1%) and girls (97.9%) (Tab 3b, 4b). As well in this case the estimated post-test probability p in boys and girls was 100% being the specificity (p2) 100%.

Discussion

Since 2013, as reported by FRONTEX, the European Agency for the Management of Operational Cooperation at the External Borders of the Member States of the European Union (www.frontex.europa.eu), the Central and Eastern Mediterranean (Libya, Italy, Malta, Tunisia, Greece, Turkey, Egypt) have become the main path by which illegal immigrants set off towards Europe. Uncontrolled immigration includes also many minors from Sub-Saharan African and South Asian countries where children are often non-registered at birth [32,33]. In fact, in the developing world, only half of the children under five years old have their births registered [33]. In sub-Saharan Africa, 64% of births go unregistered and, in South Asia, 65% of all births go unregistered [43]. In addition, since 2013, the Libyan civil war has dramatically accelerated the number of asylum-seekers and migrants crossing the Mediterranean from Libya on makeshift boats organised by traffickers [44,45].

The implications for unaccompanied children can be therefore monumental. Their official “invisibility” increases their vulnerability and children without documents proving their real age may be treated as adults in legal processes [23,32,33]. To be processed as an adult puts the child at increased risk of abuse in a system that makes no consideration for the child’s situation, age or maturity. In addition, they can be victims of abuse or hazardous work, recruited into fighting forces or forced into early marriage [1,23].

In April 2014, the European Union adopted the “Asylum, Migration and Integration Fund”, representing a commitment of over EUR 3 billion for the next seven years (2014-2020) [46]. The European Union (EU), according to the United Nations Convention on the Rights of the Child (CRC) (http://www.unicef.org/crc/), is also working towards a development of accurate protocols for assessing age of unaccompanied minors seeking asylum in European countries [23]. The CRC is a universally agreed set of non-negotiable standards and obligations which set minimum entitlements and freedoms that should be respected by governments. Article 1 of this convention effectively restricts the application of the rights contained within the CRC to those who are children, i.e. below the age of eighteen years unless under the law applicable to the child, majority is attained earlier. Therefore, a failure to recognise a person as being a child will prevent them benefitting from the rights set out in the Convention. This may have major implications for their protection, care and development and reinforces the need for State Parties to treat the subject of age assessment with due diligence and sensitivity (http://www.ohchr.org/en/professionalinterest/pages/crc.aspx).

Hence, realistic estimation of age is vital for carefully ensuring that children and juveniles are identified and treated appropriately. The techniques of age estimation may still be weakened by errors classified as technically or ethically unacceptable [32]. The age estimation methods need to minimize errors that are technically unacceptable (adults classified as minors), and possibly exclude ethically unacceptable errors (minors classified as adults). It applies especially in terms of involving the criminal responsibility of the supposed minors [32,33].

In the last few years, Cameriere at al. [25] developed a practical method for assessing adult age based on the relationship between age and the third molar maturity index (I_{3M}) which is related to the measurement of the open apices of the third molar. A cut-off value of I_{3M}=0.08 was determined. The sensitivity and specificity were 70% and 98%, respectively. In addition, the proportion of individuals correctly classified was 83%. These results encouraged also to test the cut-off value of I_{3M} = 0.08 on two samples of Albanian and Croatian subjects, respectively [47,48], from Serbia [49], from Botswana [33] and from Colombia [50]. These additional studies showed that the third molar index (I_{3M}) should be used as a useful determinant of the age of 18 not only in European countries.

Deitos et al. [51] showed that the method is suitable for estimating adulthood for forensic purposes in Brazil, although it must be applied carefully and judiciously. The analysis of 444 panoramic radiographs indicated a sensitivity of 78.3% and a specificity of 85.1%. The correct classification was of 87%. Significant differences in sexual dimorphism in the early mineralization of boys were found only for the average age with $I_{3M} \geq 0.08$, except for the range (0.7, 0.9). At the moment, the validity of the cut-off 0.08 for the correct identification of children has been tested in several populations (mainly Caucasian and South American) with, as previously mentioned, concordant results. Despite the availability of other methods based on teeth or bones developments or combined methods where both of these parameters are considered [23,52], the cut-off of 0.08 seems to demonstrate a strong consistency among different populations, providing a global instrument for adult age (< or >18) evaluation.

As regarding Libya, Putul and Azza [30] carried out the only known study on dental age estimation on a Libyan sample. They analysed the chronological age bases on third molar eruption and compared them between Libyans and Egyptians. Their study revealed that earliest third molar eruption was in the girls at 16 years of age and that eruption of the third molar was complete at 23 years of age in both the sexes.

In the present study, a sample of 307 healthy living Libyan subjects (163 female and 144 boys), aged between 14 and 22, was used to analyse the third molar development by measurements of the third molar maturity index (I_{3M}). The obtained results highlighted the importance of this supplementary analysis. They indicated correct classification in 94.5% of cases for girls and in 95.1% of cases for boys. When a cut-off of 0.09 is applied, it results in an increasing of the correct numbers of individuals correctly identified of around 3%.

The sensitivity of the test was 90.6% for girls and 90.9% for boys. The specificity was 100% for both sexes. Specificity is the probability that the test will produce a true negative result. In the reference study, the specificity of 100% has to be highlighted because this means that no false negatives have been generated in this diagnostic test and that all subjects younger than 18 years of age have been correctly classified.

Garamendi et al. [32] offered the most thorough discussion on how to combine the results in a scientifically way, as well as on the ethical dilemmas raised by the statistical variability when medical methods are used for age assessment. They argued that in the context of forensic age diagnosis, although it is important to reduce technically unacceptable errors, the primary task is to eliminate the ethically unacceptable errors. For this purpose, a combination of both skeletal and dental methods should be used to rule out the appearance of false positive results (minors being assessed as over 18 years), which will, indispensably, be on the expense of an increase in false negative results (individuals over 18 being assessed as minors).

Conclusions

According to the literature, the age estimation in the Libyan population by dental methods has not been carefully addressed and only few works focused this topic on North African region [32-34]. In addition, according to the authors' knowledge, the cut-off value by measurements of open apices has never been analysed in a Libyan sample.

The results of this study provided the first valuable method for assessing legal age of majority in a sample of Libyan subjects using a dental approach. Results are encouraging and highlighted the accuracy of I_{3M} in estimating 18 years of age in Libyan population. However, further investigations are needed in order to evaluate the accuracy of different skeletal and dental techniques in this population. Finally, further studies are needed in order to test the accuracy of I_{3M} in combination with multiple age indicators [53].

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Fig. 1. Boxplot of relationship between age and Cameriere's third molar maturity index of open apices of the mandibular right third molar in Libyan females (white) and males (gray). Boxplot shows median and inter-quartile ranges while whiskers are lines extending from box to maximum and minimum ages, excluding outliers.

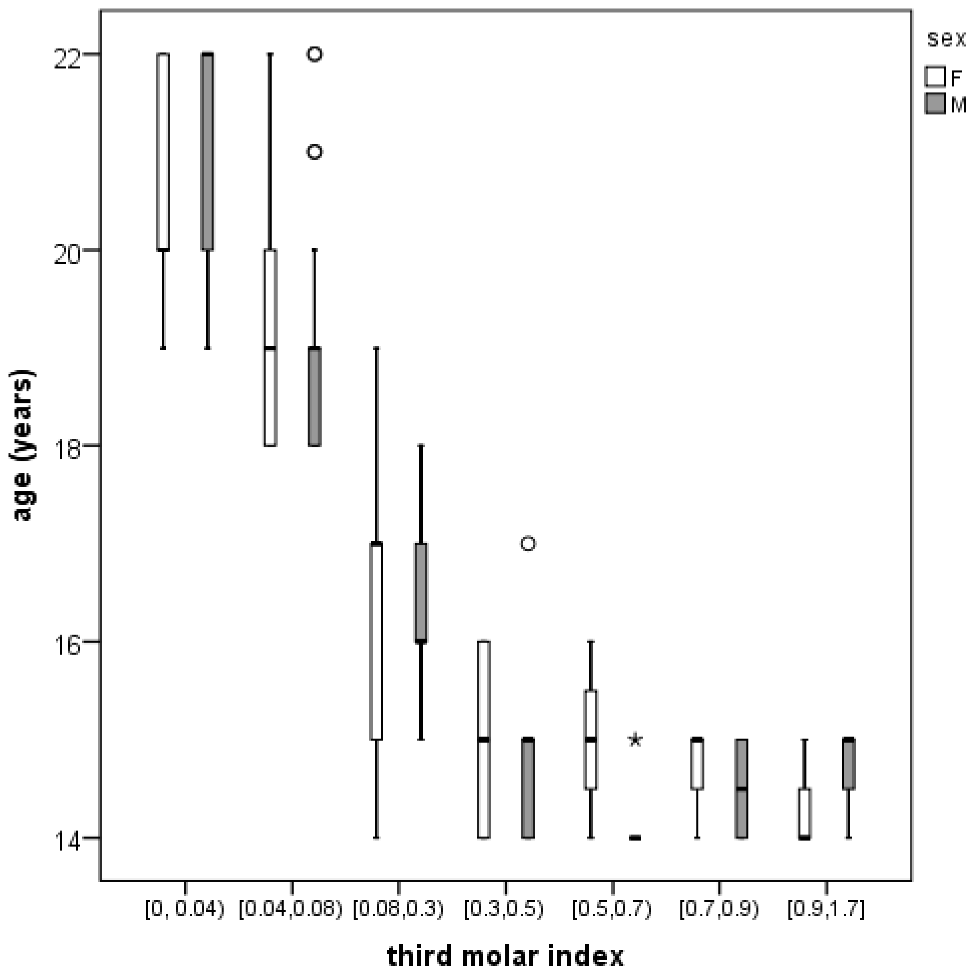

Table 1 Sample of panoramic radiographs from Libya according to sex and age categories.

Age (years)	Females	Males	Total
14	11	17	28
15	22	17	39
16	15	21	36
17	18	12	30
18	25	18	43
19	19	14	33
>20 (20-23)	53	45	98
Total	163	144	307

Table 2 Summary statistics of chronological age according to third molar maturity index (I3M): number of individuals (N), Average (AVG), Mean standard deviation (SD), Minimum value (MIN), Median (MED), maximum value (MAX).

	N	AVG	SD	MIN	MED	MAX
Females						
[0.0, 0.04)	47	20.7	1.1	19	20.5	22
[0.04, 0.08)	40	18.9	1.0	18	19.0	22
[0.08, 0.3)	53	16.4	1.2	14	17.0	19
[0.3, 0.5)	14	15.0	0.8	14	15.0	16
[0.5, 0.7)	3	15.0	1.0	14	15.0	16
[0.7, 0.9)	3	14.7	0.6	14	15.0	15
[0.9, 1.7]	3	14.3	0.6	14	14.0	15
Males						
[0.0, 0.04)	42	21.1	1.0	19	22.0	22
[0.04, 0.08)	28	19.0	1.1	18	19.0	22
[0.08, 0.3)	45	16.4	1.0	15	16.0	18
[0.3, 0.5)	10	14.9	0.8	14	15.0	17
[0.5, 0.7)	12	14.1	0.3	14	14.0	15
[0.7, 0.9)	4	14.5	0.6	14	14.5	15
[0.9, 1.7]	3	14.7	0.6	14	15.0	15

Tab 3a Contingency table describing discrimination performance of the test for males (cut-off 0.08)				Tab 3b Contingency table describing discrimination performance of the test for males (cut-off 0.09)			
			Age				
Test	>18	<18	Total	**Test**	>18	<18	**Total**
<0.08	70	0	70	**<0.09**	74	0	74
>0.08	7	67	74	**>0.09**	3	67	70
Total	77	67	**144**	Total	77	67	**144**

Table 4

Tab 4a				Tab 4b			
Contingency table describing discrimination performance of the test for females (cut-off 0.08)				Contingency table describing discrimination performance of the test for females (cut-off 0.09)			
			Age				
Test	>18	<18	Total	**Test**	>18	<18	**Total**
<0.08	87	0	87	**<0.09**	94	0	94
>0.08	9	67	76	**>0.09**	2	67	69
	96	67	**163**		96	67	**163**