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Title: **Age estimation based on a combined arteriosclerotic index**

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Abstract

This study introduces a new quantity, the combined arteriosclerotic index (CAI), which is defined as the ratio between the diameter and the longitudinal prestrain of an artery. The longitudinal prestrain has been adopted as the ratio between the in situ length and the excised length of the abdominal aorta, and is a measure of arterial elasticity. During ageing, arteriosclerosis is manifested by the loss of pretension, and by enlargement of the diameter of the artery. CAI combines these two effects. A sample of 61 female and 194 male autopsy measurements of human abdominal aortas shows that CAI correlates significantly with chronological age (R = 0.916/0.921; female/male). The sample had the following parameters: age 53±19/48±16 years; diameter of the abdominal aorta 12.4±2.2/13.4±2.1 mm; and longitudinal prestrain 1.13±0.10/1.15±0.10 (mean ± sample standard deviation; female/male). The resulting CAI was 11.2±2.7/11.9±2.6 mm. The classical linear regression model was employed for age estimation by CAI. The model gave a residual standard deviation of 7.6/6.3 years, and a 95% prediction interval range of ±15.4/12.5 years (female/male). A two-sample t-test confirmed that there are significant differences between the female and male population during ageing, reflected by CAI, unlike longitudinal prestrain. It was concluded that CAI is a suitable predictor of age at time of death, and is easily obtainable in the autopsy room.

Keywords: Aorta; ageing; age estimation; arteriosclerosis; prestrain.

Introduction

Estimating the age of cadavers of unknown identity is a standard step in forensic practice. Numerous methods, differing in principle, accuracy and precision, are frequently employed [1-5]. The approach that is used also depends on the age and the character of the remains.

Radiological methods for analyzing dental and skeletal development are accurate in children, and can be used not only for cadavers, but also for archeological cases and in the living [6-9]. The aspartic acid racemization rate (AAR) in dentine is recommended as a standard for age estimation in adults [10-12]. Methods from molecular biology for investigating telomere shortening and damage accumulation in mitochondrial DNA likewise provide a basis for age estimation [3].

Age-related changes in soft tissues can also be utilized. AAR can especially employ durable proteins like elastin. Elastic arteries and yellow ligaments have been proven to be suitable sources for this purpose [13,14]. A simple analysis can be based on the color of tissues such as the intervertebral disc, the Achilles tendon or rib cartilage [15].

It has been known for centuries that elastic arteries become stiffer with ageing [16-19]. The process which macroscopically appears as stiffening and enlargement of the artery diameter is referred to as arteriosclerosis, and has significant consequences for heart function and pressure pulse transmission. The stiffening is caused, among others, by calcium deposition, increased cross-linking (especially by non-enzymatic advanced glycation end-products) and abrupt engagement of stiff collagen fibrils within a deformation [19-21]. These processes, together with fragmentation of elastin lamellae, result in age-dependent loss of longitudinal pretension in the aorta.

It was shown in our previous studies that the magnitude of the longitudinal prestrain (the ratio between the in situ length and the ex situ length of the aortic segment) and the diameter of the abdominal aorta correlate significantly with chronological age [22,23]. However, these relationships have been found to be best fitted with the power law model. The nonlinearity results in variable variance, which is undesirable in age estimation.

This study introduces a new quantity, the combined arteriosclerotic index (CAI), which will be shown to depend linearly on chronological age. This quantity is based on the simply measurable consequences of ageing; the longitudinal prestrain and the diameter of the abdominal aorta. It will be shown that CAI is a suitable predictor of age at time of death.

Methods

This study extends our previous observations reported in [22,23]. Therefore, only basic information necessary to avoid misunderstandings will be repeated here. Detailed descriptions can be found in the references. In addition, details of the statistical approach seem to be unnecessary, and can be found in appropriate textbooks; e.g. [24].

Data describing the in situ and excised lengths of the male and female abdominal aorta, as well as the diameter (D), age, degree of atherosclerosis (ATH), and the post-mortem interval (PMI), were collected during regular autopsies of Caucasian cadavers of known age. Post-mortem usage of human tissue was approved by the Ethics Committee of the Královské Vinohrady University Hospital in Prague. No putrefied bodies were involved. The degree of atherosclerosis was examined by a pathologist and quantified on a scale from 0 to 4 [25].

Combined arteriosclerotic index – CAI

The abdominal aorta was thoroughly removed, and measurements were made of the distance between two marks in situ, and after excision. Marks were made just below the renal arteries and above the aortoiliac bifurcation. The longitudinal prestrain was quantified by means of the stretch ratio λ (1).

$$\lambda = \frac{l}{L} \tag{1}$$

Here l and L denote the in situ and ex situ lengths, respectively. Subsequently a ring was cut from the aortic segment (approx. 2 cm above the bifurcation) and it was then cut into a band, and its length was considered equal to the circumference by means of which the aortic diameter was calculated.

CAI was suggested as the ratio between the diameter and the prestrain (2). It is defined in such a way that CAI increases as D increases; and CAI increases as λ decreases. Since λ is a measure of loss of elasticity, this implies that the greater the loss of elasticity, the higher CAI will be (also for the diameter). The name of the parameter (combined arteriosclerotic index) was chosen since CAI includes the changes in both elasticity and diameter that are symptomatic for arteriosclerosis.

$$CAI = \frac{D}{\lambda} \tag{2}$$

Statistical analysis

The statistical analysis consisted of correlation, regression analysis and descriptive statistics. The correlation analysis was based on the simple linear correlation coefficient R. The regression analysis employed a classical linear model to describe the age–CAI relationship. The predictive capability of the model was evaluated via the residual standard deviation (RSD) and the prediction interval (PI). The prediction interval gives the limits in which future observation is expected with probability α (confidence level). $\alpha = 0.95$ was considered to be significant within this study. A two-sample t-test was used to reveal whether there were any differences between the male population and the female population.

Results

The data samples were higher than in our previous studied, and were increased to 194 male and 61 female individuals (the previous data is included).

Statistical data

The descriptive statistics for the total number of samples were as follows [mean ± sample standard deviation; female/male]: age 53±19/48±16 years; CAI 11.2±2.7/11.9±2.6 mm; ex situ length 76.3±12.6/78.8±11.7 mm; in situ length 85.3±10.5/89.9±11.9 mm; D 12.4±2.3/13.4±2.1; ATH 2/2 (instead of the mean, the median is used); and PMI 45±25/46±30 hours.

The statistical analysis confirmed previous findings that the retraction (the difference between the in situ length and the ex situ length), the ex situ length, the longitudinal prestrain, the diameter and the degree of atherosclerosis correlate with age. The correlation coefficients are presented in Table 2. It was found that CAI provides an even higher correlation with age than the diameter and the prestrain. The in situ length of the abdominal segment of the aorta does not show significant dependence on age (R = 0.192/-0.069 female/male).

The results of the t-test (Table 1; significance indicated by *) show that the quantities based solely on the lengths of the aortic segments (in situ and ex situ length, retraction and λ) do not differ significantly with respect to gender. The quantities based on aortic diameter (CAI), however, do differ significantly.

Regression model

The results of the regression analysis are depicted in Fig. 1. The regression parameters are given in the caption to the figure. The analysis revealed RSD = 7.6/6.3 years; and PI = 15.4/12.5 years in the female/male group. The regression lines intersect the experimental data almost uniformly, which suggests that an appropriate model was used. The data was also tested to find whether l, L, λ, D and CAI follow a Gaussian distribution. They were adjusted for age, and the Shapiro-Wilk test was performed (confidence level 0.05). The results suggested that the hypothesis of normality should not be rejected.

Discussion

The combined arteriosclerotic index, defined as the ratio between the diameter of the excised artery and its longitudinal prestrain (2), was proposed as a measure of ageing. It was shown that the linear model is suitable to fit the data that had been collected. The correlation between age and CAI (R = 0.916/0.921 female/male) was even higher than in the case of the diameter–age and prestrain–age dependencies. The data presented here suggests that the age-dependent distribution of the in situ length, the ex situ length, the retraction and the prestrain in the abdominal aorta are not gender-specific. As was to be expected the diameter depends on sex. This dependence is replicated in CAI.

The close correlation between CAI and chronological age makes CAI suitable for use in simple and instant age estimation. The main advantage of the method is the possibility to obtain an estimation straightaway in the autopsy room.

Comparison with existing methods

The reviews [2,26] state that age estimation methods giving a sample standard error of estimate higher than 7 years cannot provide an accurate basis for routine forensic application. From this point of view, the CAI method gives accuracy just at the limit (RSD = 7.6/6.3 years; F/M).

The CAI method provides better or comparable accuracy in comparison with methods recommended for age estimation in adults (an evaluation of dental morphology, including histological features); the standard errors published in [2] range from 4-10 years. An evaluation of bone histology (standard error of estimate 5-12 years) also gives comparable results. However, AAR determines age with a standard error of 1.5-4 years [2] which is superior to the CAI method.

In comparison with our previous studies, which employed nonlinear regression models for the prestrain–age and diameter–age relationships, the proposed combined arteriosclerotic index depends linearly on age. This fact gives the advantage of almost constant variance. Moreover, CAI yields more accurate results (the 95% prediction

intervals in the power law models employed in [22] yielded ranges of 30.5-62.3 and 25.3-57.0 years for the mean prestrain in the female population and in the male population, respectively).

Limits of the CAI method

Putrefied bodies were excluded from this study, since putrefaction can affect the biomechanical properties of an artery wall. In such cases, the method cannot be used. Previous studies have shown that PMI does not correlate significantly with prestrain [22,23]. This result is replicated in the CAI method (correlation coefficient R = 0.148/0.024 female/male). Nevertheless, we may expect that a longer PMI than was used in our study may lead to a different observation.

The method introduced here was derived from observations operating with adults. This fact limits our findings and the possible application of regression parameters. Cadavers with tortuous or aneurysmatic abdominal aorta were also not included, since the definition of prestrain is questionable in such cases.

The principal limit of CAI is its non-repeatability. Once the aortic segment has been excised, measurement of the in situ length cannot be repeated. This drawback can be overcome by including another elastic artery as a control sample. The carotid, iliac and femoral arteries have been proven to be longitudinally prestrained and are easily obtainable for measurements [27-29]. Unfortunately, suitable studies for comparison have not yet been carried out.

Biomechanics

Although the aim of our study is to introduce a new method for estimating age at time of death we should, at least briefly, clarify what CAI measures from the biomechanical viewpoint. It seems to be obvious that it is something like age-dependent deformation or elasticity. However, the true deformation must be dimensionless, and the elasticity has to be in Pascals (force per unit area, which is the unit of the Young modulus in Hooke's law). Nevertheless, CAI has a dimension of length.

Consider a cylindrical surface (a prestrained artery) of length l and diameter d. The longitudinal constraint is released upon artery excision, and the artery shrinks to length L, and expands to diameter D. The true deformation of the surface area can be defined as $\pi \cdot d \cdot l/(\pi \cdot D \cdot L)$. It is clear that CAI is only a fragment of this biomechanical information (area ratio = d/CAI). It is highly probable that the area ratio will depend on age, due to overall stiffening during ageing. Unfortunately, we are not able to measure the diameter in situ with desirable accuracy. It is also not clear whether the area ratio depends linearly on age since the prestrain, which is a dimensionless deformation measure, depends non-linearly on age [22,23]. Finally, it should be noted that this was a somewhat simplified interpretation, since the cylindrical geometry of an artery is also prestrained in the circumferential direction (this strain is usually called "residual", and is manifested when a radial cut of an arterial ring is made as an abrupt opening to a sector [30,31]). We therefore prefer to use CAI, although its biomechanical interpretation is not as straightforward as in case of the area ratio.

Conclusion

The study has confirmed that the retraction of the abdominal aorta, the ex situ length and the longitudinal prestrain correlate with chronological age, and do not differ significantly with respect to gender. The combined arteriosclerotic index was found to depend linearly on age. This study has suggested that CAI is a suitable candidate for simple and instantaneous age estimation.

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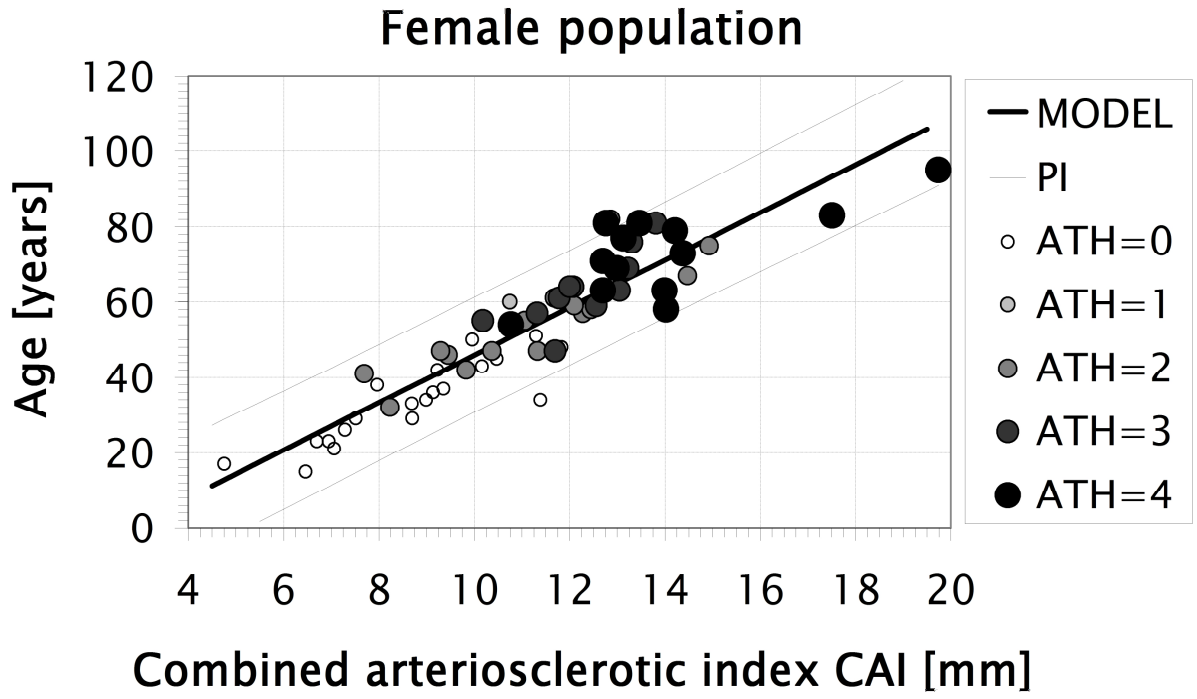
Table 1. Measured data (mean ± sample standard deviation) sorted with respect to decades of age. Abbreviations and corresponding units: age [years]; number of observations – n [-]; combined arteriosclerotic index – CAI [mm]; ex situ length – L [mm]; in situ length – l [mm]; difference between in situ and ex situ length (i.e. retraction) – ΔL [mm]; longitudinal prestrain – λ [-]; diameter – D [mm]; degree of atherosclerosis – ATH [-]; and post mortem interval – PMI [hours]. Indices F and M indicate sex. The † symbol indicates that the median was used. A two-sample t-test was used to evaluate the differences between the mean in male and female populations in age groups older than 19 years. Differences significant with a probability 0.95 or higher are indicated by *.

Age	<20	20-29	30-39	40-49	50-59	60-69	69<
n_F	2	6	7	11	11	12	12
n_M	2	30	32	32	47	39	12
CAI_F	5.6 ±1.2	7.4* ±0.7	9.1 ±1.1	10.1* ±1.2	11.6* ±1.2	12.6* ±1.1	14.4* ±2.1
CAI_M	6.7 ±0.4	8.1* ±0.8	10.1 ±1.0	11.3* ±1.1	13.2* ±1.1	14.2* ±1.2	15.9* ±1.4
L_F	61.0 ±2.8	61.7* ±4.1	69.3 ±12.2	77.2 ±8.8	78.2 ±9.4	79.3 ±8.6	84.8 ±16.4
L_M	71.0 ±5.7	68.7* ±8.6	75.5 ±9.1	80.6 ±10.0	81.7 ±10.8	83.4 ±13.0	83.3 ±12.9
l_F	86.5 ±4.9	80.3* ±4.9	80.7 ±11.3	86.6 ±8.3	86.1 ±9.4	86.0 ±7.6	87.8 ±16.7
l_M	96.5 ±9.2	88.9* ±12.5	90.4 ±9.8	92.8 ±9.8	89.5 ±12.5	88.9 ±13.2	87.9 ±13.5
ΔL_F	25.5 ±2.1	18.7 ±2.2	11.4* ±2.8	9.5* ±3.4	7.9 ±2.1	6.7 ±2.5	3.1 ±2.4
ΔL_M	25.5 ±3.5	20.2 ±5.7	14.8* ±3.8	12.2* ±4.2	7.8 ±3.6	5.5 ±2.6	4.6 ±2.6
λ_F	1.42 ±0.02	1.30 ±0.04	1.17 ±0.07	1.13 ±0.06	1.10 ±0.03	1.09 ±0.04	1.04 ±0.03
λ_M	1.36 ±0.02	1.29 ±0.07	1.19 ±0.06	1.16 ±0.06	1.10 ±0.04	1.07 ±0.04	1.06 ±0.03
D_F	7.9 ±1.8	9.6 ±1.0	10.6* ±1.1	11.3* ±1.0	12.8* ±1.2	13.6* ±1.0	14.9* ±2.0
D_M	9.1 ±0.7	10.5 ±0.8	12.0* ±1.0	13* ±1.0	14.4* ±1.0	15.1* ±1.1	16.8* ±1.4
ATH_F	$0^{\dagger}$	$0^{\dagger}$	$0^{\dagger}$	$2^{\dagger}$	$2^{\dagger}$	$3^{\dagger}$	$4^{\dagger}$
ATH_M	$0^{\dagger}$	$0^{\dagger}$	$0^{\dagger}$	$2^{\dagger}$	$3^{\dagger}$	$4^{\dagger}$	$4^{\dagger}$
PMI_F	29 ±10	47 ±27	32 ±18	42 ±21	55 ±24	57 ±35	52 ±33
PMI_M	32 ±18	34 ±16	44 ±27	56 ±34	48 ±31	43 ±33	59 ±47

Table 2 Correlation coefficients R. The first row indicates pairs of quantities used in the calculation.

Sex	ΔL–age	L–age	λ–age	D–age	ATH–age	CAI–age	PMI–CAI
F	-0.863	0.557	-0.840	0.878	0.783	0.916	0.148
M	-0.804	0.386	-0.820	0.888	0.792	0.921	0.024



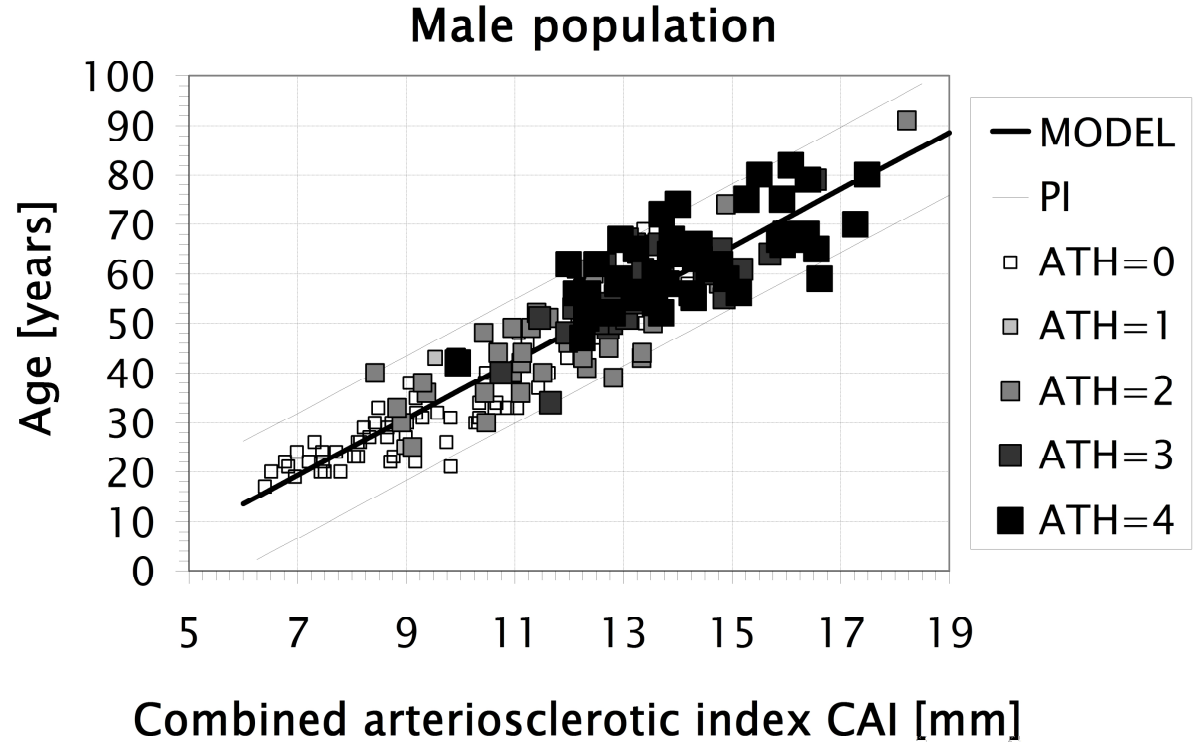


Figure 1. Regression model. The classic linear model was employed in the regression analysis; Age = *a**CAI + *b*. The parameters were computed as follows: *a* = 6.307/5.776; *b* = -17.17/-21.13 (female/male). The observation points are presented with respect to the degree of atherosclerosis. The prediction intervals are depicted with dashed lines. They show the intervals in which future observations will fall with probability equal to 0.95. The result is ±15.4 and ±12.5 years around the regression model in the female and male population, respectively.