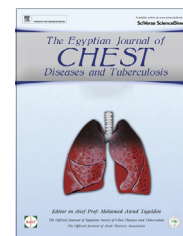


The Egyptian Society of Chest Diseases and Tuberculosis  
Egyptian Journal of Chest Diseases and Tuberculosis

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ORIGINAL ARTICLE

# Estimated lung age in healthy North African adults cannot be predicted using reference equations derived from other populations

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Received 15 September 2013; accepted 24 September 2013

Available online 13 October 2013

## KEYWORDS

Lung age;  
Smoking cessation;

**Abstract** *Introduction:* Interpretation of “lung age” data relies upon comparison of the chronological lung age (CLA) with the estimated lung age (ELA) predicted from published reference equations [7–10].

*Abbreviations:* ATS, American Thoracic Society; BMI, Body Mass Index; CLA, chronological lung age; Delta LA, CLA minus ELA; ELA, estimated lung age; ERS, European Respiratory Society; FEF<sub>x</sub>, forced expiratory flow when x% of forced vital capacity has been exhaled; FEV<sub>1</sub>, first second forced expiratory volume; FVC, forced vital capacity; LLN, Lower-Limit-of-Normal; MMEF, maximal mid-expiratory flow; NHANES-3, Third National Health and Nutrition Evaluation Survey; PEF, peak expiratory flow; SD, standard deviation; ULN, Upper-Limit-of-Normal; 95% CI, 95% confidence interval.

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☆ Peer review under responsibility of The Egyptian Society of Chest Diseases and Tuberculosis.



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Spirometry;  
North Africa;  
Reference equation;  
Limits of normal

*Aim:* To test the applicability of the published reference equations in healthy non-smoker Tunisian aged 19–90 years.

*Population and methods:* Published reference equations were applied to the spirometry results of 540 adults (364 women). Two methods of comparison were applied: (i) Determination, according each equation, of the percentages of subjects having a  $\text{deltaLungAge} (= \text{ELA} - \text{CLA}) > \text{Upper-Limit-of-Normal (ULN)}$ . (ii) Bland and Altman comparison, for the same age range as in the corresponding study, between CLA and ELA.

*Results:* The mean  $\pm$  SD (95% confidence interval) of the total sample CLA and height were  $48.8 \pm 13.1$  (47.7–49.9) years and  $164 \pm 10$  (163–165) cm. (i) The percentages of healthy subjects with a  $\text{deltaLungAge} > \text{ULN}$  varied from 1% (Newbury) to 64% (Hansen) in men, and from 20% (Yamaguchi) to 51% (Hansen, Morris and Temple) for women. (ii) Mean  $\pm$  SD ELA was significantly underestimated by  $17 \pm 19$  years (Hansen), by  $12 \pm 23$  years (Morris and Temple) and was significantly overestimated by  $4 \pm 19$  years (Newbury). Mean  $\pm$  SD ELA from Yamaguchi et al. [10] was not statistically different from the CLA ( $1 \pm 14$  years).

*Conclusion:* The published reference equations did not reliably predict CLA data in the Tunisian population. Awaiting the establishment of reliable equation proper to North African population, we recommend the use of the Yamaguchi et al.'s [10] reference equations.

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## Introduction

Smoking, shown to be detrimental to health for many years [1–4], has an adverse effect on the first second forced expiratory volume ( $\text{FEV}_1$ ) throughout a lifetime, reducing the maximal  $\text{FEV}_1$  achieved, bringing forward the age of onset of decline in  $\text{FEV}_1$ , and hastening the rate of decline [5]. The single most useful intervention to improve lung function in smokers, with or without, chronic obstructive pulmonary disease is smoking cessation. One way to increase the quit rate in smokers could be to communicate about the lung function results in a manner that is easily understood and stimulates the desire to quit.

Estimated lung age (ELA) is an estimate that uses the observed spirometric variable (often  $\text{FEV}_1$ ) of a smoker to calculate the approximate age of a healthy non-smoker with the same spirometric variable based on predicted values [6]. ELA reference equations were developed as an aid for smoking cessation counseling and the concept has been explored in several recent publications [7–11]. Interpretation of “lung age” data relies upon comparison of the chronological lung age (CLA) values with ELA predicted from available reference equations [7–10]. To our knowledge, only four studies have published equations predicting ELA [7–10]. These equations were first developed by Morris and Temple in 1985 for the USA population [7] using earlier American predictive equations for spirometry published in 1971 [12]. Four models of ELA reference equations were developed and the most relevant model to determine ELA values was the one using  $\text{FEV}_1$  [7]. In 2010, two other reference equations were developed by Newbury et al. [8] and by Hansen et al. [9], respectively, for the South Australian and USA populations. In 2012, Yamaguchi et al. [10] have developed for the Japanese population, novel regression equations predicting lung age from varied spirometric parameters.

These four studies used limited methodology:

*Low sample size* (i.e., only 125 subjects were included in the Australian study [8]),

*Sample may not be representative of a normal population* (i.e., 79% of Morris and Temple [7] subjects were from 2 church groups in rural USA, subjects of the South Australian study [8] maintain a high level of fitness and have occasional occupational exposures to smoke when attending fires),

*Use of old data* (i.e., predictive equations [12] for Morris and Temple study that are the basis of the Morris ELA equations [7] are 41 years old; the Third National Health And Nutrition Evaluation Survey (NHANES-3) [13,14] data that Hansen et al. [9] equations are based on are now approximately 20 years old),

*Skewed age distribution* (i.e., results of Morris and Temple study [7] are biased towards younger ages and the age distribution is strongly skewed to the right with over 30% of subjects aged between 20 and 30 years),

*Use of old equipment and application of old spirometric methods* (i.e., Morris and Temple's [7] and Hansen et al.'s [9] results were calculated using equipment (respectively, stead-wells and dry rolling-seal spirometers) and methods that give lower results than those currently recommended by the American Thoracic and European Respiratory Societies (ATS/ERS) [15–17]). The study of Newbury et al. [8] predates the first ATS guidelines on spirometry [18] and not the last ones of 2005 [15–17]),

*Mathematical and statistical flaws* (i.e., Morris and Temple [7] method having a couple of mathematical and statistical flaws, lung age was an estimated value based on the population mean [8], application of a circular argument [9] with equations predicting the actual mean age of the subjects from whom they were derived),

*Different models of reference equations* (i.e., different spirometric parameters were included in the reference equations: only  $\text{FEV}_1$  [7,8], only ratio between  $\text{FEV}_1$  and forced vital capacity (FVC) [9], various spirometric parameters such as FVC,  $\text{FEV}_1$ ,  $\text{FEV}_1/\text{FVC}$ , peak expiratory flow (PEF), forced expiratory flow when  $x\%$  of FVC has been exhaled ( $\text{FEF}_x$ ,  $\text{FEF}_{50}$  and  $\text{FEF}_{25}$  and maximal mid-expiratory flow (MMEF)) [10]),

*Linear function and ageing* (i.e., no reliable grounds for supporting the idea that the relationship between lung ageing and various spirometric parameters can be approximated by a linear function [4]),

*Wide variation in ELA* (i.e., quite wide variability of spirometry results of normal healthy subjects (80–120% predicted) [8], and consequently wide variation in ELA, exists [8]), and

*Data interpretation* (i.e., only two authors [7,10] have proposed an algorithm for judging the abnormality from spirometry ELA with presentation of a recommended sequence to interpret ELA [7] or a recommendation to use the Upper-Limit-of-Normal and Lower-Limit-of-Normal (ULN, LLN, respectively) [10], as recommended for spirometry [19]).

These methodological shortcomings explain some discrepancies in the findings. Indeed, Newbury et al. [8] have shown that Morris and Temple [7] equations significantly underestimate CLA in both never-smokers and smokers and that ELA by new Australian equations [8] produces ELAs that are approximately 20 years greater than does the Morris and Temple [7] equations, for South Australian never-smoking and current smoking males. Of greater concern is that, in the smoker subgroup of Newbury et al. [8], the ELA mean by the Morris and Temple [7] equations was 12 years lower than the CLA mean, indicating a ‘protective’ effect of tobacco smoking. A couple of authors, however, questioned whether the ELA was truly useful as a tool for motivating the cessation of smoking [20,21]. They asserted that the ELA from the method of Morris and Temple [7] entirely disregarded the variability of FEV<sub>1</sub> in normal subjects, thus causing a physiologically serious flaw, i.e., the ELA of a normal person whose FEV<sub>1</sub> is below the reference value but above the LLN is forcibly estimated to be older than his/her CLA, though the ELA of this person should be equal to the CLA. This happens because the ELA is calculated by counting back the regression equation predicting the reference value, but not the LLN, of FEV<sub>1</sub>.

How to evaluate “spirometric” ELA and what method is approvable? This question was asked by some authors in 2011 [22], in order to promote the development of ethnic-specific ELA regression equations in various races. The need for normal values specific to North African populations has been demonstrated for several physiological parameters [23–29]. So, the applicability and the reliability of published ELA reference equations [7–10] should be assessed as regards the North African adult population, in order to avoid erroneous clinical interpretation of ELA data in this population.

The aim of the present paper is to test the applicability of the previously published ELA reference equations [7–10] in healthy adult North African population, represented by Tunisian subjects (the null hypothesis is that there will be no difference between CLA and ELA mean values).

## Design and methods

### Study design

We performed this cross-sectional study over a one year period (February 2011–January 2012) in the 2 Functional Exploration Laboratories at the Occupational Medicine Group and at the Farhat HACHED Hospital of Sousse

(altitude < 100 m), Tunisia. Approval for the study was obtained from the Hospital Ethics Committee and a written informed consent was obtained from all study participants.

### Study population

Target population consists of a sample of subjects aged 19 years and more, living in Sousse, Tunisia. Subjects were recruited from local workers visiting the Functional Exploration Laboratory at the Occupational Medicine Group of Sousse, or from the staff of the Faculty of Medicine and the Farhat HACHED Hospital in Sousse, as well as acquaintances of people involved in the study. The Functional Exploration Laboratory at the Occupational Medicine Group of Sousse offers several explorations (electrocardiogram, visual test, audiogram and spirometry) as a routine service to local workers (subjects or patients). Approximately 5000 spirometry procedures are performed annually and workers are addressed by occupational physicians for several reasons: record review of employment, working in a risk position (i.e., dust, glue, etc.), further investigation of a complaint (i.e., dyspnea, cough, etc.), control of a known respiratory illness, and cigarettes or narghile smoking. The main reason of performing spirometry was undergoing the general health screening examination. The Functional Exploration Department of Farhat HACHED Hospital offers several explorations (spirometry, plethysmographie, bronchial hyper-responsiveness test, exercise testing, etc.) as a specialized service to local subjects or patients. Approximately 8000 cardio-respiratory procedures are performed annually and subjects are addressed by physicians for several reasons, especially cardio-respiratory complaints. Reasons for performing spirometry as well as other clinical details were not analyzed further.

### Sample size

It was calculated using the following predictive equation [30]:  $n = (Z^2 \cdot p \cdot q) / \Delta^2$ , where  $n$  is the number of subjects necessary,  $Z$  is the 95% confidence level ( $Z = 1.96$ ),  $p$  is the prevalence of healthy non-smoker adult population aged more than 19 years and free from disease, which is of the order of 60% in Tunisia according to a recent study [31],  $q$  is equal to “ $1 - p$ ” and  $\Delta$  is the precision (= 4.5%). According to this formula the number of subjects required was 455.

### Inclusion and non-inclusion criteria

Only healthy and “normal” subjects aged between 19 and 90 years, having complete records and a technically acceptable and reproducible spirometry maneuvers were included in the study. A healthy and “normal” person is defined as one in whom there is [32,33]: (i) no presence of acute and no past chronic disease of the respiratory system; (ii) no major respiratory disease, such as congenital anomalies, destructive type of pneumonia or thoracic surgery in past medical history; (iii) no systemic disease which may directly or indirectly influence the respiratory system and general state of health (e.g., cardiovascular, neuromuscular, skeletal or renal disease); (iv) no history of upper respiratory tract infection during three weeks prior to investigation; (v) no underweight, no severe or massive obesity; (vi) Lifelong non-smokers (cigarettes and/or narghile) or no more than incidental smoking experience. Added non-inclusion criteria were respira-

tory work exposure and an abnormal spirometric data ( $FEV_1$  and/or FVC and/or  $FEV_1/FVC$  ratio  $< LLN$ ).

#### Collected data

CLA from identity card, sex (men, women), anthropometric data (age, weight, height, Body Mass Index (BMI)), parity, spirometric data (FVC,  $FEV_1$ , PEF, MMEF,  $FEF_{75}$ ,  $FEF_{50}$ ,  $FEF_{25}$ ,  $FEV_1/FVC$  ratio), ELAs from the published studies [7–10]. The study protocol was as follows: welcome and provision of an information sheet; completion of medical questionnaire, and anthropometric and spirometric measures.

#### Medical questionnaire, tobacco use evaluation

Data were collected using a simplified non-validated version of a medical questionnaire recommended for epidemiological research [34]. It was composed of questions (mainly closed questions, usually dichotomous) put to the subjects in local Arabic dialect. It was used to assess subject characteristics: cigarette smoking and narghile use [35–37], medical, surgical, and gynecologic-obstetric histories and medication use.

#### Anthropometric measurements

The decimal age (accuracy to 0.1 years) was calculated from the date of measurement and the date of birth [38]. Due to the failure of software to compute decimal age as the difference between test date and birth date, age was taken as the number of complete years from birth to the date of the study. Standing height and weight were measured using a stadiometer and expressed to the nearest centimeter and kilogram, respectively. Depending on calculated BMI ( $kg/m^2$ ), we distinguished between [39]: underweight ( $BMI < 18.5$ ), normal weight ( $18.5 \leq BMI < 25$ ), overweight ( $25 \leq BMI < 30$ ) and obese ( $BMI \geq 30$ ). The latter was either moderate ( $30 < BMI < 35$ ), severe ( $35 \leq BMI < 40$ ), or massive ( $BMI \geq 40$ ).

#### Spirometry function tests

Spirometry was carried out in the sitting position, and a nose clip was applied. To avoid the problem of variability due to different

technicians and devices [15], all tests were performed, between 9.00 am and 1.00 pm, by only two qualified persons (one person at each site). All subjects performed spirometry on a dry rolling seal spirometer (Spida5; Micro Direct, Inc. 803 Webster Street Lewiston, ME 04240). The flow sensor of the spirometer, which was calibrated daily with a 3-liter syringe (to ensure performance), is a hot-wire anemometer, and the range of air flow linearity is 0.01–16.00 l/s with an accuracy of  $\pm 3\%$  between 0.01 and 12.00 l/s. Spirometry was performed according to the recent international recommendations [15]. The spirometric data (FVC (l);  $FEV_1$  (l);  $FEF_x$  (l/s), PEF (l/s),  $FEV_1/FVC$  ratio (absolute value)) were expressed at “body temperature, barometric pressure saturated with water vapor” [15–17]. LLN for spirometric data was calculated from the local spirometric norms [26–28]. Any observed value for  $FEV_1$ , FVC and  $FEV_1/FVC$  ratio lower than its corresponding LLN was considered abnormal.

#### Data analysis

##### Expression modes of results

The Kolmogorov–Smirnov test was used to analyze distribution of variables [30]. When the distribution is normal and the variances are equal, the results are expressed by their mean  $\pm$  standard deviation (SD) and 95% confidence interval (95% CI). If the distribution is not normal, the results are expressed by their medians (1<sup>st</sup>–3<sup>rd</sup> quartiles). The chi-2 test was used to compare percentages. Preliminary descriptive analysis included frequencies for categorical variables (sex) and mean  $\pm$  SD (or median (1<sup>st</sup>–3<sup>rd</sup> quartiles)) for continuous ones (anthropometric and spirometric data).

##### Published ELA reference equations (Box 1)

Morris and Temple [7] have developed 6 reference equations models for the USA population aged 20–84 years. Newbury et al. [8] have developed 2 reference equations models for the South Australian population aged 25–74 years. Hansen et al. [9] have developed only one reference equation model for the USA population aged 20–80 years.  $FEV_1/FVC$  (%) for normal USA never-smoking adults were independent of ethnicity and sex [40,41] and equal to  $98.8 - 0.25 \times \text{Age (years)} - 1.79 \times \text{FVC (l)}$ . Yamaguchi et al. [10] have developed 2 reference equations models for the Japanese population aged 25–87 years.

#### Box 1. Published ELA reference equations

Morris and Temple [7]

##### Men

$$ELA = 2.331 \times \text{Height (cm)} - 40.000 \times \text{Observed FVC (l)} - 169.640$$

$$ELA = 1.130 \times \text{Height (cm)} - 31.250 \times \text{Observed FEV}_1 \text{ (l)} - 39.375$$

$$ELA = 0.411 \times \text{Height (cm)} - 22.222 \times \text{Observed MMEF (l/s)} + 55.844$$

##### Women

$$ELA = 1.887 \times \text{Height (cm)} - 41.667 \times \text{Observed FVC (l)} - 118.833$$

$$ELA = 1.401 \times \text{Height (cm)} - 40.000 \times \text{Observed FEV}_1 \text{ (l)} - 77.280$$

$$ELA = 0.787 \times \text{Height (cm)} - 33.333 \times \text{Observed MMEF (l/s)} + 18.367$$

Newbury et al. [8]

$$\text{Men: } ELA = 1.56 \times \text{Height (cm)} - 33.69 \times \text{Observed FEV}_1 \text{ (l)} - 85.62$$

$$\text{Women: } ELA = 1.33 \times \text{Height (cm)} - 31.98 \times \text{Observed FEV}_1 \text{ (l)} - 74.65$$

Hansen et al. [9]

$$\text{Men and women: } ELA = CLA + 3 \times (\text{predicted-observed}) FEV_1/FVC \text{ ratio (percentage)}$$

Yamaguchi et al. [10]

$$\text{Men: } ELA = 209.195 - 0.455 \times \text{Height (cm)} - 11.521 \times \text{Observed FEV}_1 \text{ (l)} - 0.602 \times \text{Observed FEV}_1/FVC \text{ (\%)} + 1.956 \times \text{Observed FEF}_{50} \text{ (l/s)}$$

$$\text{Women: } ELA = 234.441 - 0.792 \times \text{Height (cm)} - 7.295 \times \text{Observed FEV}_1 \text{ (l)} - 0.610 \times \text{Observed FEV}_1/FVC \text{ (\%)} + 0.301 \times \text{Observed PEF (l/s)} + 2.647 \times \text{Observed FEF}_{50} \text{ (l/s)}$$

### Comparison of ELA data

Four methods were applied.

- (i) Student's *t*-test was used to compare CLA versus ELA.
- (ii) We have determined, according to each reference equation [7–10], the number (%) of healthy subjects with ELA “clinically and significantly” higher than the CLA. According to Yamaguchi et al. [10], ELA is considered as “clinically and significantly” higher than the CLA, when the delta lung age (delta LA = ELA – CLA) is higher than the ULN equal to 13.4 years in men or 15.0 in women.
- (iii) We have determined according to each reference equation and for the same age range as in the corresponding studies [7–10], the number (%) of healthy subjects with ELA below zero or over 110 years.
- (iv) Graphical comparison with published reference equations [7–10]: CLAs were compared with ELAs calculated from the published reference equations [7–10] for the same age range as in the corresponding study, in several ways. First, our CLA values were compared with ELA calculated from the published reference equations [7–10] using scatter plots and paired *t* tests. Second, as proposed by Bland and Altman [42], comparisons between CLA and ELA were performed by means of the limits of agreement, where individual differences (delta LA) were plotted against the corresponding mean value. From these data, limits of agreement were then calculated (mean delta LA  $\pm$  1.96 SD) [42]. The reference equation that provides the limits of agreement closest to zero will be the most appropriate for our population.

Analyses were carried out using Statistica software (Statistica Kernel version 6; StatSoft, Paris, France). Significance was set at 0.05 level.

## Results

### Descriptive data

#### Non-inclusion criteria

An initial sample of 667 adult volunteers was examined. Non-inclusion criteria, presented in detail in E.Table 1 (Supplementary data) were found in 129 subjects.

#### Anthropometric and spirometric data

The dependent variable (CLA) was normally distributed (Kolmogorov–Smirnov = 0.066,  $p < 0.05$ ). Fig. 1 shows the age and sex distribution of the 540 healthy subjects (176 men). We note a significantly large number of women included in the age ranges between 19.0 and 60.0 years. Table 1 exposes the anthropometric and spirometric data of the included healthy subjects. When compared with men, women were significantly more aged, significantly shorter and had a significantly higher BMI. Expressed in percent of local predicted values, all women observed spirometric data were significantly higher than those of men, except for the FEV<sub>1</sub>/FVC ratio and FEF<sub>50</sub> variables.

### Analytical data

#### ELA data

Table 2 exposes the comparison between CLA and ELA determined from the published reference equations.

ELAs from Newbury et al.'s reference equations are significantly different from the CLAs (overestimated by 19 years in men, underestimated by 4 years in women and overestimated by 4 years in the total sample).

ELAs from Hansen et al.'s reference equation are significantly lower than the CLAs (underestimated by 17, 16 and 16 years, respectively for men, women and the total sample).

ELAs from Yamaguchi et al.'s reference equations are significantly different from CLAs for men and women (respectively, overestimated by 9 years and underestimated by 5 years). However, the total sample ELA was not statistically different from the CLA.

ELAs from Morris and Temple's reference equation model using FEV<sub>1</sub> are significantly lower than the CLAs for women and the total sample (underestimated by 18 and 11 years, respectively). However, the men's ELA was not statistical different from the CLA.

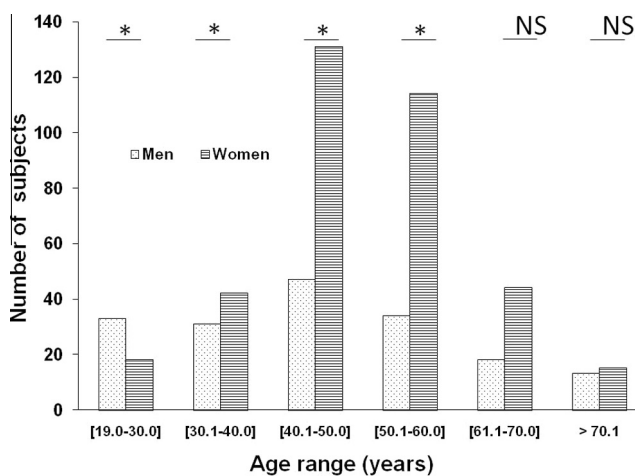
ELAs from Morris and Temple reference equation model using FVC are significantly different from CLAs for men and women (respectively, overestimated by 19 years and underestimated by 8 years). However, the total sample ELA was not statistically different from the CLA.

ELAs from Morris and Temple reference equation model using MMEF are significantly lower than the CLAs (underestimated by 11, 29 and 23 years, respectively for men, women and the total sample).

ELAs from Morris and Temple reference equation model using the mean of the 3 above models are significantly different from CLAs for men, women and total sample (respectively, overestimated by 3 years, underestimated by 18 years and underestimated by 11 years).

#### Number of healthy subjects with abnormal ELA

Table 3 presents, according to the published reference equations, the number (%) of healthy Tunisian subjects with



**Figure 1** Distribution of the total sample by sex and age ranges. \*  $p < 0.05$  (Chi-2): men vs. women. NS: non significant.

**Table 1** Anthropometric and spirometric data.

	Men ( <i>n</i> = 176)	Women ( <i>n</i> = 364)	Total sample ( <i>n</i> = 540)
Age (Year) <sup>a</sup>	45.4 ± 15.5 (43.1–47.8)	50.5 ± 11.4* (49.3–51.6)	48.8 ± 13.1 (47.7–49.9)
Height (cm) <sup>a</sup>	166 ± 8 (165–167)	163 ± 11* (162–164)	164 ± 10 (163–165)
Weight (kg) <sup>a</sup>	74 ± 12 (72–76)	73 ± 12 (72–74)	73 ± 12 (72–74)
Body Mass Index (kg/m <sup>2</sup> ) <sup>a</sup>	26.8 ± 3.8 (26.2–27.4)	27.5 ± 3.5* (27.1–27.9)	27.3 ± 3.6 (27.0–27.6)
Parity (numerical) <sup>a</sup>		5 ± 3 (4–5)	
Normal weight <sup>b</sup>	58 (33%)	88 (24%)	146 (27%)
Overweight <sup>b</sup>	81 (46%)	178 (49%)	259 (48%)
Moderate obesity <sup>b</sup>	37 (21%)	98 (27%)	135 (25%)
1 <sup>st</sup> second forced expiratory volume (FEV <sub>1</sub> , l) <sup>a</sup>	3.22 ± 0.62 (3.13–3.32)	2.97 ± 1.00* (2.87–3.07)	3.05 ± 0.90 (2.98–3.13)
FEV <sub>1</sub> (% predicted) <sup>a</sup>	97 ± 11 (96–99)	114 ± 21* (112–116)	109 ± 20 (107–110)
Forced vital capacity (FVC, l) <sup>a</sup>	3.81 ± 0.72 (3.70–3.92)	3.51 ± 1.19* (3.38–3.63)	3.61 ± 1.07 (3.52–3.70)
FVC (% predicted) <sup>a</sup>	95 ± 11 (93–96)	114 ± 22* (112–117)	108 ± 21 (106–110)
FEV <sub>1</sub> /FVC (absolute value) <sup>a</sup>	0.85 ± 0.06 (0.84–0.86)	0.85 ± 0.06 (0.84–0.86)	0.85 ± 0.06 (0.84–0.85)
Peak expiratory flow (PEF, l/s) <sup>a</sup>	7.32 ± 1.58 (7.08–7.56)	6.45 ± 2.23* (6.22–6.68)	6.73 ± 2.08 (6.56–6.91)
PEF (% predicted) <sup>a</sup>	88 ± 19 (85–91)	100 ± 27* (97–103)	96 ± 25 (94–98)
Forced expiratory flow when 25% of FVC has been exhaled (FEF <sub>25</sub> , l/s) <sup>a</sup>	1.99 ± 0.74 (1.88–2.10)	1.93 ± 1.16 (1.81–2.05)	1.95 ± 1.04 (1.86–2.04)
FEF <sub>25</sub> (% predicted) <sup>a</sup>	111 ± 36 (106–116)	123 ± 71* (116–130)	119 ± 62 (114–124)
Forced expiratory flow when 50% of FVC has been exhaled (FEF <sub>50</sub> , l/s) <sup>a</sup>	4.69 ± 1.37 (4.48–4.89)	4.28 ± 1.54* (4.12–4.44)	4.41 ± 1.50 (4.28–4.54)
FEF <sub>50</sub> (% predicted) <sup>a</sup>	103 ± 27 (99–107)	108 ± 30 (105–111)	107 ± 29 (104–109)
Forced expiratory flow when 75% of FVC has been exhaled (FEF <sub>75</sub> , l/s) <sup>a</sup>	6.68 ± 1.55 (6.45–6.91)	5.80 ± 2.42* (5.55–6.05)	6.09 ± 2.21 (5.90–6.27)
FEF <sub>75</sub> (% predicted) <sup>a</sup>	92 ± 20 (89–95)	102 ± 37* (98–106)	99 ± 33 (96–102)
Maximal mid-expiratory flow (MMEF, l/s) <sup>a</sup>	4.04 ± 1.13 (3.87–4.21)	3.75 ± 1.60* (3.58–3.91)	3.84 ± 1.47 (3.72–3.97)
MMEF (% predicted) <sup>a</sup>	102 ± 24 (98–105)	113 ± 40* (109–117)	110 ± 36 (107–113)

*p* < 0.05 (\*Student-test, <sup>†</sup>Chi-2): men vs. women.

<sup>a</sup> Data are mean ± SD (95% confidence interval).

<sup>b</sup> Data are number (%).

**Table 2** Comparison between chronological lung age (CLA) and estimated lung age (ELA) calculated from published reference equations.

	Men ( <i>n</i> = 176)	Women ( <i>n</i> = 364)	Total sample ( <i>n</i> = 540)
CLA (Year)	45.4 ± 15.5 (43.1–47.8)	50.5 ± 11.4 (49.3–51.6)	48.8 ± 13.1 (47.7–49.9)
ELA (Year) from Newbury et al. reference equation	64.4 ± 17.9 (61.8–67.1) <sup>a</sup>	46.8 ± 21.8 (44.6–49.1) <sup>a</sup>	52.6 ± 22.2 (50.7–54.4) <sup>a</sup>
ELA (Year) from Hansen et al. reference equation	28.4 ± 20.6 (25.3–31.5) <sup>a</sup>	34.0 ± 18.8 (32.0–35.9) <sup>a</sup>	32.2 ± 19.5 (30.5–33.8) <sup>a</sup>
ELA (Year) from Yamaguchi et al. reference equation	54.8 ± 9.2 (53.4–56.2) <sup>a</sup>	45.1 ± 12.1 (43.8–46.3) <sup>a</sup>	48.2 ± 12.1 (47.2–49.3)
ELA (Year) from Morris and Temple reference equations models using:			
First second forced expiratory volume	47.2 ± 16.6 (44.8–49.7)	32.2 ± 28.9 (29.2–35.2) <sup>a</sup>	37.1 ± 26.5 (34.9–39.3) <sup>a</sup>
Forced vital capacity	64.4 ± 24.2 (60.8–68.0) <sup>a</sup>	42.7 ± 34.3 (39.2–46.2) <sup>a</sup>	49.8 ± 33.0 (47.0–52.6)
Maximal mid-expiratory flow	34.2 ± 24.4 (30.6–37.9) <sup>a</sup>	21.7 ± 47.4 (16.8–26.6) <sup>a</sup>	25.8 ± 41.7 (22.3–29.3) <sup>a</sup>
The mean of the 3 above models	48.6 ± 19.1 (45.8–51.5) <sup>a</sup>	32.2 ± 34.7 (28.6–35.8) <sup>a</sup>	37.6 ± 31.5 (34.9–40.2) <sup>a</sup>

Data are mean ± SD (95% confidence interval).

<sup>a</sup> *p* < 0.05 (paired-test): CLA vs. ELA.

ELA “clinically and significantly” higher than the CLA. These percentages varied from 1% (Newbury et al.) to 64% (Hansen et al.) in men, and from 20% (Yamaguchi et al.) to 51% (Hansen et al. and Morris and Temple model using MMEF), in women. For the total sample the lowest percentages are seen with Yamaguchi et al. and Newbury et al., respectively, 14% and 16%. Table 3 presents also the number (%) of healthy subjects with ELA (calculated for the same age range as in the corresponding study) below zero or over 110 years. We conclude that when applied in our population,

- (i) Yamaguchi et al.’s reference equations give no persons with an ELA below zero or over 110 years;
- (ii) Newbury et al.’s reference equations give 3% of healthy women with an ELA below zero.
- (iii) Hansen et al.’s reference equation gives 6% of healthy men and 3% of healthy women with an ELA below zero.
- (iv) Morris and Temple’s reference equations give the highest percentage of healthy subjects with an ELA below zero, especially in women.

**Table 3** Number (percentage) of Tunisian healthy adults having an abnormal estimated lung age (ELA).

ELA derived from reference equation of	Men		Women		Total sample	
<i>ELA "clinically and significantly" higher than the chronological lung age (men/women:176/364)</i>						
Newbury et al.	2 (1%)		85 (23%)		87 (16%)	
Hansen et al.	113 (64%)		185 (51%)		298 (55%)	
Yamaguchi et al.	4 (2%)		74 (20%)		78 (14%)	
Morris and Temple models using:						
1st second forced expiratory volume	17 (10%)		175 (48%)		192 (36%)	
Forced vital capacity	6 (3%)		139 (38%)		145 (27%)	
Maximal mid-expiratory flow	75 (43%)		186 (51%)		261 (48%)	
The mean of the three above models	18 (10%)		154 (42%)		172 (32%)	
<i>ELA (calculated for the same age range as in the corresponding study) below zero or over 110 years</i>						
	Below zero	Over 110	Below zero	Over 110	Below zero	Over 110
Newbury et al. (men/women: 148/348)	0 (0%)	0 (0%)	11 (3%)	0 (0%)	11 (2%)	0 (0%)
Hansen et al. (men/women: 171/360)	10 (6%)	0 (0%)	11 (3%)	0 (0%)	21 (4%)	0 (0%)
Yamaguchi et al. (men/women: 154/355)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Morris and Temple models (men/women:171/361) using:						
1st second forced expiratory volume	0 (0%)	0 (0%)	63 (17%)	0 (0%)	63 (15%)	0 (0%)
Forced vital capacity	1 (1%)	5 (3%)	50 (14%)	2 (1%)	51 (10%)	7 (1%)
Maximal mid-expiratory flow	11 (6%)	0 (0%)	106 (29%)	0 (0%)	117 (22%)	0 (0%)
The mean of the three above models	0 (0%)	0 (0%)	82 (23%)	0 (0%)	82 (15%)	0 (0%)

### Graphical comparison with published reference equations

Figs. 2 and 3 show, for the same age range as in the corresponding study, CLA individually plotted against the corresponding ELA values predicted from reference equations of Morris and Temple [models using FEV<sub>1</sub> (Fig. 2A) or FVC (Fig. 2B) or MMEF (Fig. 2C) or the mean of FEV<sub>1</sub> and FVC and MMEF (Fig. 2D)], of Hansen et al. (Fig. 3A), of Newbury et al. (Fig. 3B) and of Yamaguchi et al. (Fig. 3C). As can be seen, the data showed a wide disparity compared with the identity line.

Figs. 4 and 5 show, for the same age range as in the corresponding study, the Bland and Altman comparisons between CLA and ELA predicted from the published reference equations. There was a systematic bias between the CLA and ELA values for most of these equations; that is to say, the difference with CLA increased as the ELA increased. This was particularly evident for the equations of Morris and Temple (models using FEV<sub>1</sub> or MMEF or the mean of FEV<sub>1</sub>, FVC and MMEF) (Fig. 4A, C and D, respectively), or from Hansen et al. (Fig. 5A). The correlation between delta LA and mean values was also significant for the Morris and Temple's equation model using FVC ( $p < 0.001$ , Fig. 4B), and Newbury et al.'s equation ( $p = 0.001$ , Fig. 5B). On the other hand, the correlation between mean  $\Delta$ LA and mean values was not significant for the Yamaguchi et al.'s reference equations ( $p = 0.16$ , Fig. 5C). Second, as can be deduced from Figs. 4 and 5, there was a difference between CLA and ELA determined from these published reference equations, which was often statistically significant. Indeed, mean  $\pm$  SD ELA was significantly underestimated by  $23.0 \pm 37.3$  years ( $p < 0.001$ ), by  $16.6 \pm 18.7$  years ( $p < 0.001$ ), by  $11.8 \pm 22.8$  years ( $p < 0.001$ ), by  $11.4 \pm 27.1$  years ( $p < 0.001$ ) and by  $1.9 \pm 13.1$  years ( $p = 0.009$ ), respectively, with the reference equations of Morris and Temple model using MMEF, (Fig. 4C); of Hansen et al. (Fig. 5A); of Morris and Temple model using FEV<sub>1</sub> (Fig. 4A);

of Morris and Temple model using the mean of FEV<sub>1</sub>, FVC and MMEF, (Fig. 4D); and of Yamaguchi et al. (Fig. 5C). ELA was significantly but slightly overestimated by  $2.8 \pm 19.3$  years ( $p < 0.01$ ) with the reference equations of Newbury et al. (Fig. 5B). For the total sample data, mean  $\pm$  SD ELA derived from Morris and Temple model using FVC (Fig. 4B) was not significantly different from CLA (mean  $\pm$  SD =  $-0.7 \pm 29.0$  years;  $p = 0.673$ ). However, when examined separately, ELA derived from Morris and Temple model using FVC was significantly underestimated by  $7.8 \pm 29.1$  years ( $p < 0.001$ ) and overestimated by  $18.8 \pm 18.7$  years ( $p < 0.001$ ), respectively in men and women.

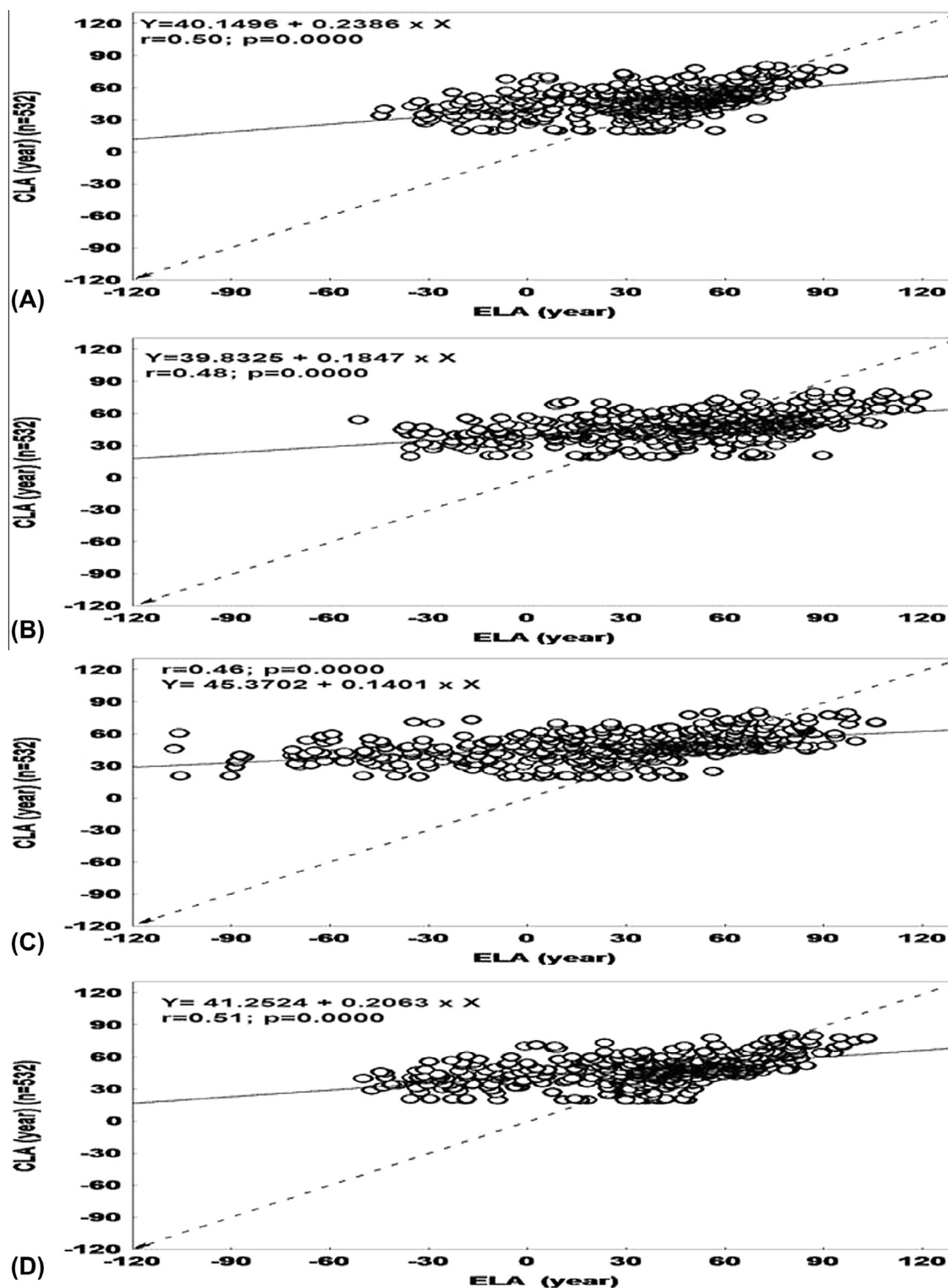
### Discussion

Published and locally applied spirometric reference equations for ELA did not reliably predict CLA data in 540 healthy adult subjects representing a sample of the Tunisian population. So, we can reject the null hypothesis that we would see no difference in the means of the CLA and ELA produced by the published reference equations [7–10]. Our results strongly suggest that existing lung age equations [7–10] are in need of review.

### Methodology discussion

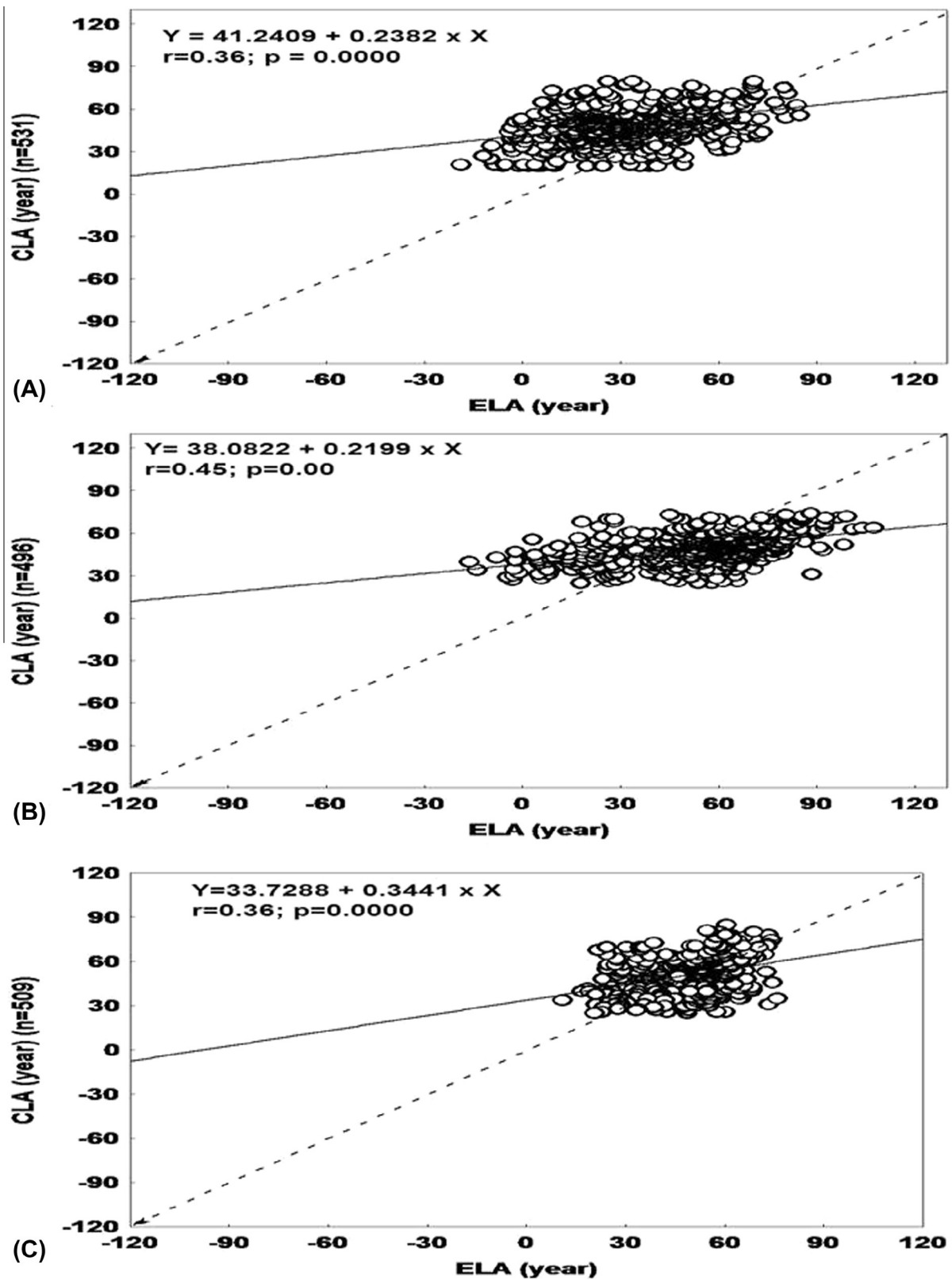
#### Study design

As for the studies aiming to publish ELA reference equations [7–10], ours was not a random population sample. Some caution should be warranted when interpreting the results of cross-sectional studies in volunteers, because of a possible selection bias and cohort effects [43]. Thus, longitudinal studies analyzed by appropriate statistical models are necessary to correctly describe the functional changes associated with age [18]. This was recently demonstrated by Newbury et al. [44] who conclude that ELA using six predictive equations span-

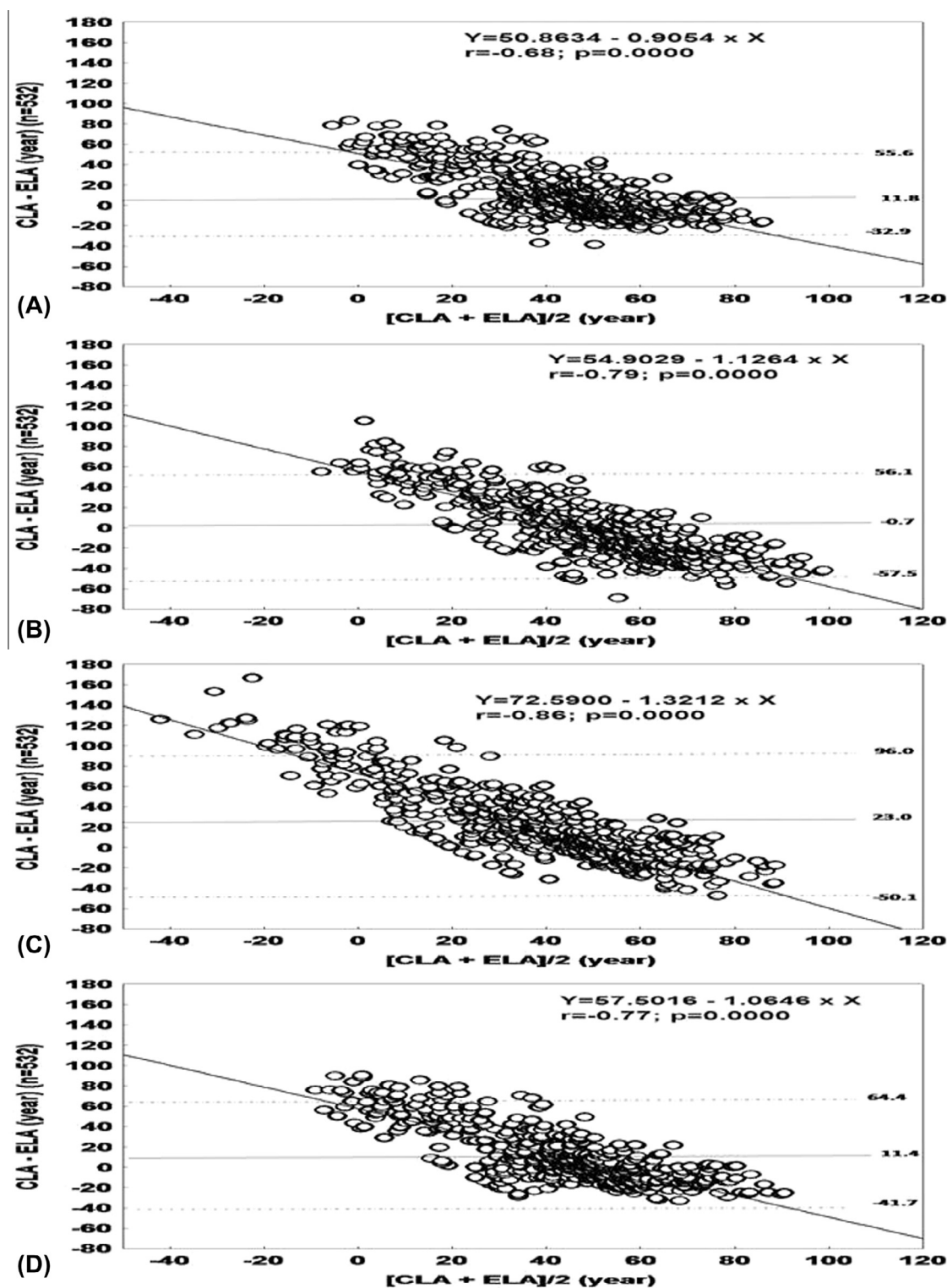


**Figure 2** Comparison, for the same age range as in the corresponding study, of chronological lung age (CLA) with estimated lung age (ELA) determined from reference equations of Morris and Temple models using: first second forced expiratory volume (FEV<sub>1</sub>) (Fig. A), Forced vital capacity (FVC) (Fig. B), Maximal mid-expiratory flow (MMEF) (Fig. C), The mean of FEV<sub>1</sub> and FVC and MMEF (Fig. D).  $n$  = number of subjects having the same age range as in the corresponding study;  $r$ : correlation coefficient;  $p$ : probability. Solid line (—): regression line; Dashed line (-----): identity line.

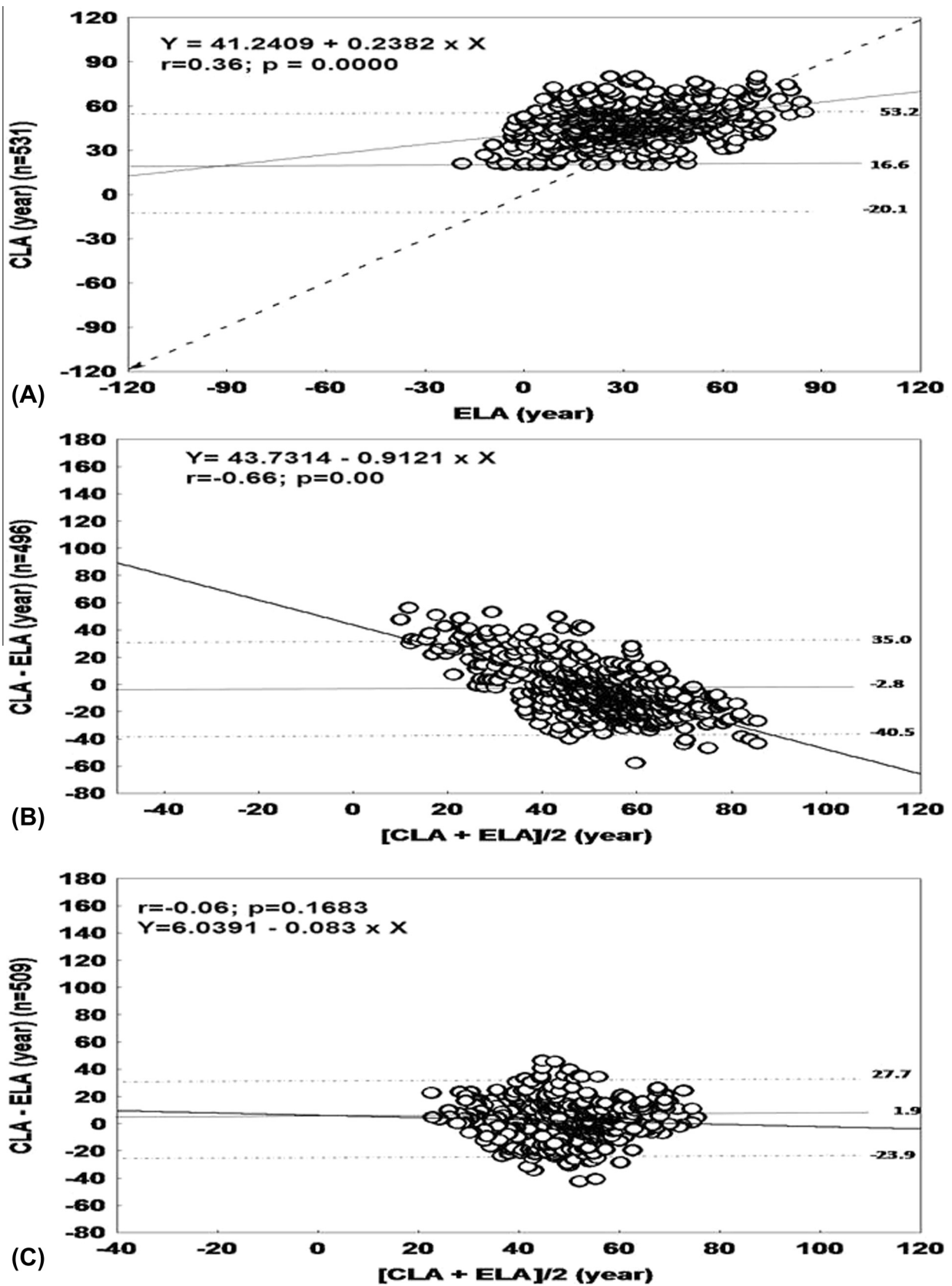




**Figure 3** Comparison, for the same age range as in the corresponding study, of chronological lung age (CLA) with estimated lung age (ELA) determined from reference equations of: Hansen et al. (Fig. A), Newbury et al.(Fig. B), Yamaguchi et al. (Fig. C),  $n$  = number of subjects having the same age range as in the corresponding study;  $r$ : correlation coefficient;  $p$ : probability. Solid line (—): regression line; Dashed line (-----): identity line.



**Figure 4** The Bland and Altman representation, for the same age range as in the corresponding study, of chronological lung age (CLA) with estimated lung age (ELA) determined from reference equations of Morris and Temple models using: first second forced expiratory volume ( $FEV_1$ ) (Fig. A), Forced vital capacity (FVC) (Fig. B), Maximal mid-expiratory flow (MMEF) (Fig. C), The mean of  $FEV_1$  and FVC and MMEF (Fig. D),  $r$ : correlation coefficient;  $p$ : probability;  $n$  = number of subjects having the same age range as in the corresponding study. — Mean; .....: mean  $\pm$  1.96  $\pm$  SD; / : regression line.



**Figure 5** The Bland and Altman representation, for the same age range as in the corresponding study, of chronological lung age (CLA) with estimated lung age (ELA) determined from reference equations of: Hansen et al. (Fig. A), Newbury et al. (Fig. B), Yamaguchi et al. (Fig. C),  $r$ : correlation coefficient;  $p$ : probability;  $n$  = number of subjects having the same age range as in the corresponding study. —: Mean; .....: mean  $\pm$  1.96  $\pm$  SD; —: regression line.

ning 50 years showed differences attributable to cohort and period effects. Although no statistical methods were used to choose the volunteers, the number of subjects studied [38] and the fact that many private or government-owned firms in different areas of Sousse (Tunisia) were included give a reasonable degree of confidence in the data.

#### Population source

Our study design (prospective study) and our recruitment mode were similar to those of the Japanese study [10] (E.Table 2, supplementary data). The three other studies were retrospective types (E.Table 2, supplementary data). The greater percentage (85%) of our participants was sorted from those undergoing the general health screening examination at the Functional Exploration Laboratory at the Occupational Medicine Group of Sousse.

#### Sample size and characteristics

In a recent study [38] aiming to establish the number of local subjects required to validate published reference equations, it was found that at least 150 men and 150 women would be necessary to validate the reference equations to avoid spurious differences due to sampling error. Our sample size ( $n = 540$ ) appears to be satisfactory since the calculated one is 455 subjects. It was higher than the sample size of one study [8] ( $n = 125$ ), but was smaller than the sample sizes of other studies [ $n = 988$  [7];  $n = 7428$  [9] and  $n = 8015$  [10]] (E.Table 2, supplementary data). Like in the Japanese study [10], our sample was dominated by women (respectively, 68.8% and 67.4%). In the 3 other studies, the percentage of included women were, 47.7% [7], 52.8% [8] and 59.0% [9] (E.Table 2, supplementary data). Like the South Australian sample [8], ours was evenly age-stratified (Fig. 1). The Morris and Temple [7] sample was strongly skewed to the right with over 30% of subjects in the youngest 10 year age bracket.

#### Applied inclusion and non-inclusion criteria

As it is recommended for such epidemiological study, we have included only healthy adults as defined by international guidelines [32,33]. The applied inclusion and non-inclusion criteria were different from one study to another (E.Table 3, supplementary data). Only Yamaguchi et al. [10] have assessed smoking durations or habits or exposure to second-hand smoke or environmental exposures.

#### Spirometry measurements

As for the study of Yamaguchi et al. [10], and differently to the other three published studies [7–9] (E.Table 3, supplementary data), we have applied the recent international guidelines for spirometry [15–17].

*Comparison with published reference equation:* to our knowledge, the present study is the first one to have tested the reliability of the published ELA reference equations [7–10] on healthy adult population having a different race (the Arab race) than the races explored in other studies (E.Table 3, supplementary data). In addition, in our study, all Morris and Temple's reference equations [7] are checked, which has been done for the first time in the literature. All previous studies [8–10] have focused on Morris's and Temple reference equation model's using FEV<sub>1</sub> [7].

#### Results discussion

There was a significant difference between CLA and ELA determined from the published reference equations [7–10] (Figs. 4 and 5). These results provide us important information. First, the null hypothesis that we would see no difference in the means of the CLA and ELA produced by the published reference equations [7–10] is rejected. Second, the application of the published ELA reference equations [7–10] as regards the Tunisian adult population; induce erroneous clinical interpretation of ELA data in this population.

How can we explain these differences? There are several possible reasons for this significant result and it is possible that all play a role (E.Tables 2 and 3, supplementary data).

#### Methodological reasons

*Low sample size.* The South Australian study [8] sample size ( $n = 125$  subjects) (E.Table 2, supplementary data) is lower than the recommended sample size of 150 men and 150 women [15]. A relatively large number of subjects ( $n = 300$ ) is necessary to be confident that a significant difference between the published reference equations and the values from the local community does not exist [6]. The South Australian study [8] sampling error can explain a part of the spurious differences.

*Sample representation and race.* There have been recommendations to perform spirometric measurements on a representative sample of healthy subjects [16]. Seventy-nine percent of subjects included in the Morris and Temple study [7] were from two church groups in rural USA. The doctrines of these churches forbid tobacco smoking, the intake of alcohol or caffeine as well as advocating a vegetarian diet. The population sample of the South Australian study [8] was drawn from the broad rural community, targeting non-smokers with no history of lung disease [45]. But, retained subjects maintain a high level of fitness and contrarily have occasional occupational exposures to smoke when attending fires [8]. These samples [7,8] cannot be described as representative of a 'normal' current day population and may not be representative of a normal population. Participants in the Yamaguchi et al.'s [10] study were sorted from those undergoing the general health screening examination at the Japanese red cross kumamoto health care center. Several studies [15–17] have demonstrated ethnic differences in pulmonary function, and ELA reference equations based on American [7,9], South Australian [8] or Japanese [10] populations may not perform well on North African population [26,28].

*Use of old data.* Guidelines for spirometry [15] recommend that reference equations should be derived from a 'relevant' population and should be updated at least every 10 years. These recommendations should equally apply to ELA reference equations. Predictive equations [12] for the American study [7] that are the basis of the Morris and Temple ELA reference equations are 41 years old. The NHANES-3 [13,14] data that Hansen et al.'s [9] equations are based on were collected between 1988 and 1994, and are also now approximately 20 years old. The cohort effect suggests that a 40 year-old today will not be the same as someone of the same age 40 years ago due to demographic and environmental differences [32].

*Skewed age distribution.* Results of Morris and Temple study [7] are biased towards younger ages and the age distribution is strongly skewed to the right with over 30% of subjects aged between 20 and 30 years. One of the strengths of the South Australian sample [8] was it is evenly age-stratified, resulting in reference equations that are equally relevant across the whole age range.

*Use of old equipment and application of old spirometric methods.* Respiratory testing equipment and procedures have been progressively refined over the last 41 years in line with recommendations that have been regularly updated by the ATS/ERS [15–17]. According to these societies, ideally spirometric reference equations should be derived from a population similar to the individual subject using the same kind of instrument and testing procedure [15–17]. The Morris and Temple's [7] and Hansen et al.'s [9] results were calculated using equipment (respectively, steady-wells and dry rolling-seal spirometers) and methods that give different results than those currently recommended by the ATS [19]. The study of Newbury et al. [8] predated the first ATS guidelines on spirometry [18]. Only Yamaguchi et al. [10] predated recent ATS/ERS spirometry guidelines [15–17].

*Other, not evaluated factors, known to influence lung function.* Other factors that also influence lung function throughout life (gestational age at birth [46], genetics [47], childhood infections [48], and environmental factors such as air quality and workplace exposures [49]) were not evaluated. Also, the relationship between ELA and parity, a particular factor in developing nations [4.3 in Tunisia [50]; 1.6 in Europe and North America [51]] has not been analyzed. In previous studies [26–28], some authors have demonstrated that high parity accelerates pulmonary function decline: FEV<sub>1</sub> decreased by 84 ml when parity increased by one unit. For developing countries, ELA for women should take into consideration the parity level.

#### *Statistical reasons*

*Mathematical and statistical flaws.* Although the original method proposed by Morris and Temple [7] has undoubtedly contributed toward the smoking-cessation program [52], it had a couple of mathematical and statistical flaws. Since the ELA from spirometric measurements is absolutely important when considering the enlightenment concerning chronic obstructive pulmonary disease and smoking cessation, it is indispensable to establish the reliable method allowing estimation of spirometric lung age [22,53]. In addition, in the South Australian study [8], lung age was an estimated value based on the population mean. This has potential difficulties when predicting values for individuals. This was demonstrated by the large SD of ELA for never-smokers (18.66 years) or current-smokers (22.52 years) derived from the South Australian ELA reference equations [8]. Hansen et al. [9] have applied a circular argument: equation predicts the actual mean age of the subjects from whom they were derived.

*Different models of ELA reference equations.* Morris and Temple [7] and Newbury et al. [8] have included only FEV<sub>1</sub> in their reference equations and have presented different models for men and women. Hansen et al. [9] have chosen to include only FEV<sub>1</sub>/FVC ratio that is independent of ethnicity and gender

[40,41]. They have justified their choice by the fact that in normal American populations, the FEV<sub>1</sub>/FVC ratio has much less variability than absolute measures of other spirometric volumes or flows [54]. It is indistinct whether the ELA can be reliably predicted simply from one spirometric parameter, often FEV<sub>1</sub> [53]. For that reason, Yamaguchi et al. [10] have included various spirometric parameters as explanatory variables.

*Linear function and ageing.* There are no reliable grounds for supporting the idea that the relationship between the ageing lung and various spirometric parameters can be approximated by a linear function. Kohansal et al. [4] demonstrated that, in the male, peak of FEV<sub>1</sub> or FVC would be attained at an age between 20 and 25 years-old and then would decline with age, but, in the female, full lung growth would be achieved earlier than in the male. In practice, reference equation should be derived by valid and biologically meaningful statistical models, taking into account the dependence of lung function with age and height [16]. Furthermore, the backward calculation of age from the regression equation for the reference value of FEV<sub>1</sub> may not be allowed in a statistical sense [22,53,55]. For example, when the original method of Morris and Temple [7] is applied, the ELA of a person with measured FEV<sub>1</sub> beyond ULN results in being remarkably young (sometimes, below zero), while that of a person with FEV<sub>1</sub> below LLN is estimated as being very elderly (sometimes, over 100) [53] (table 3).

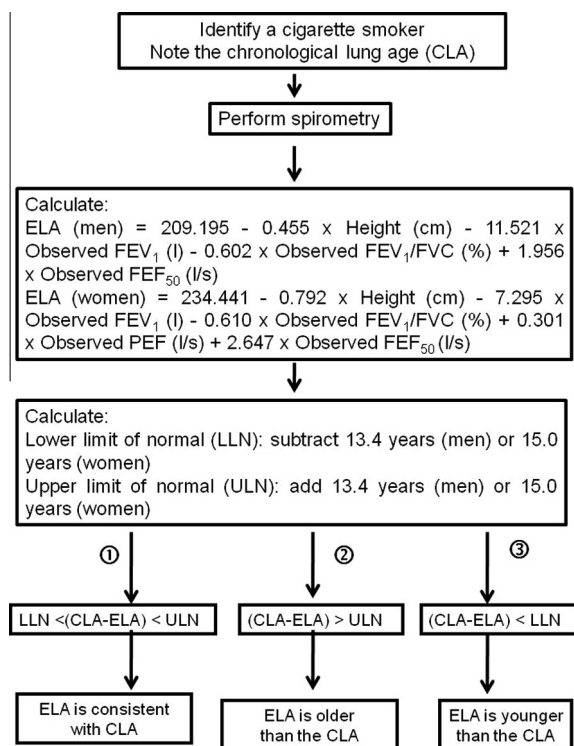
*Wide variation in ELA.* The variability of South Australian spirometry results of normal healthy subjects was quite wide (80–120% predicted) and consequently a wide variation in ELA exists [8].

*Data interpretation ways.* Publications on reference equations should include explicit definitions of the ULN and LLN, or provide information to allow the reader to calculate a lower range [16]. Only two authors [7,10] have proposed an algorithm for judging the abnormality from spirometry ELA. Morris and Temple [7] have presented a recommended sequence to interpret ELA and Yamaguchi et al. [10] have recommended the use of ULN and LLN [19].

#### *How to evaluate "spirometric" ELA for North African population? What method is approvable?*

First of all, our results strongly suggest that existing ELA reference equations [7–10] are in need of review. These results further confirm the need to use modern lung age equations which will provide a stronger message in smoking cessation counseling. That is to say that their use in North African population induces erroneous clinical interpretation of ELA. So, and as it is recommended by international guidelines [16] that encourage investigators to develop and publish specific spirometric reference equations for healthy individual, it is time to set the North African ELA reference equations.

Second, from the published reference equations [7–10], and waiting the establishment of reliable equation proper to North African population, we recommend the use of the equations presented by Yamaguchi et al. [10] for healthy subjects aged 25–87 years. Our recommendation is based on the following reasons.



**Figure 6** Five-step procedure for judging the abnormality of estimated lung age (ELA) in North African population. For abbreviations, see list of abbreviations.

- (i) The Yamaguchi et al.'s [10] study, the only prospective one, concerns the biggest sample size (E.Table 2, supplementary data).
- (ii) The study methodology and the statistical approach [10] to determine ELA reference equations are very satisfying (E.Table 2, supplementary data).
- (iii) Since their reference equations include  $FEV_1/FVC$  and  $FEF_x$  as explanatory variables, they are expected to be sensitive to the functional abnormality caused by the interstitial lung pathology, as well [10].
- (iv) Yamaguchi et al. [10] were the only authors who have presented a clear algorithm for judging the abnormality from spirometry-derived lung age (E.Table 2, supplementary data).
- (v) When applied in our Tunisian healthy adult population, the total sample CLA was equal to ELA determined from the Japanese equations [10] (Table 2). Also, among the four published studies [7–10], Yamaguchi et al. [10] gives the lowest percentage of subjects with ELA “clinically and significantly” higher than the CLA and no subjects with an ELA lower than zero or over 110 years (Table 3). Moreover, the Japanese study [10] provides the lowest delta LA ( $1.9 \pm 13.1$  years) (Fig. 5C).

Our suggestion is in line with the international guidelines where it is recommended that the reference equation that provides the sum of residuals (observed–predicted computed for each adult subject) closest to zero will be the most appropriate for a local laboratory [16]. In practice, we would recommend

the five-step procedure, explained in Fig. 6, when judging the abnormality in ELA.

## Conclusion

In conclusion, published and locally applied spirometric reference equations for ELA did not reliably predict CLA data in Tunisian healthy adult subjects. Among the published reference equations [7–10], and waiting the establishment of reliable equation proper to North African population, we recommend the use of the equation presented by Yamaguchi et al. [10]. Since the genetic and phenotypic differences among the populations are considerable and affect the prediction, we recommend

- (1) To establish novel North African regression equations allowing prediction of the reference value of ELA and its normal limits using data from a large number of healthy Tunisian never-smokers with normal spirometric measurements; and
- (2) To validate the developed equations using data obtained from another group of healthy never-smokers with normal spirometry and groups of subjects with deteriorating pulmonary function such as chronic obstructive pulmonary disease patients.

## Conflict of interest

None.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ejcdt.2013.09.018>.

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