Brief Communication
Diagnostic Immunology

Ann Lab Med 2023;43:82-85
https://doi.org/10.3343/alm.2023.43.1.82
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Establishment of Reference Intervals of Cytokeratin 19 Fragment Antigen 21-1 in Korean Adults

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Cytokeratin 19 fragment antigen 21-1 (CYFRA 21-1) is useful for predicting and monitoring non-small cell lung cancer prognosis. We established reference intervals (RIs) of CYFRA 21-1 in Korean adults, including those older than 60 years. Data of 4,098 apparently healthy subjects (age range, 20–87 years) were analyzed after excluding those with a history of malignancy, high tumor marker concentrations (except CYFRA 21-1), and/or abnormal findings on a chest computed tomography scan through medical chart review. After removing two outliers, RIs of CYFRA 21-1 were determined using data of 4,096 subjects based on the non-parametric method (2.5th and 97.5th percentiles) according to CLSI guidelines EP28-A3c. The subjects were divided into two and four groups according to sex and age (20–40, 41–50, 51–60, and >60 years), respectively, and the median CYFRA 21-1 concentration was compared between the groups. The RI of CYFRA 21-1 was 0.66–3.84 ng/mL, applicable to both men and women. Regardless of sex, the CYFRA 21-1 concentration increased with age, suggesting that age-dependent RIs of CYFRA 21-1 should be applied. Rather than using a single RI provided by the manufacturer, the RI of CYFRA 21-1 should be continually verified and established in each clinical laboratory.

Key Words: CYFRA 21-1, Reference interval, Age, Sex, Korean, Adult

Received: March 29, 2022
Revision received: April 28, 2022
Accepted: August 3, 2022

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Lung cancer is the second most common cancer following breast cancer in women and the most common cause of cancer-related deaths worldwide [1]. The most common type of lung cancer is non-small cell lung cancer (NSCLC), which accounts for more than 80% of lung cancers and includes adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [2, 3]. As lung cancer has a poor prognosis, early detection of cancer progression is important [4]. Cytokeratin 19 fragment antigen 21-1 (CYFRA 21-1) is a soluble fragment of cytokeratin 19 that serves as a serum tumor marker. It is the major component of keratin-containing intermediate filaments in epithelial cells, and its usefulness in predicting and monitoring the prognosis of NSCLC has been demonstrated [4-9].

Tumor markers, including CYFRA 21-1, are affected by various factors, such as sex, age, and ethnicity [10-12]. While applying a single reference interval (RI) regardless of sex or age may reduce the sensitivity and specificity of tumor markers, the single RI of CYFRA 21-1 provided by the manufacturer is used in most clinical laboratories [11, 12]. To the best of our knowledge, only one Korean study has attempted to establish an RI of CYFRA 21-1, and the study included adults aged 20–60 years [12]. Korea is an aging society with a large elderly population, and the burden of cancer care is increasing [13]. Therefore, it is imperative to establish an RI of CYFRA 21-1 for all adults, including those older than 60 years. We aimed to establish RIs of CYFRA 21-1 in Korean adults, including those older than 60 years, according to the CLSI guidelines [14].

In total, 4,604 medical charts were collected from subjects who visited the Chung-Ang University Medical Center (CAUMC), Seoul, Korea, for a general health examination between Septem-

ber 2020 and February 2021. The Institutional Review Board (IRB) of the CAUMC approved the study protocol (2107-003-19373) and waived the requirement of obtaining informed consent from the study subjects according to the IRB policy. Based on medical chart reviews, subjects who met the following criteria were excluded: (1) having a history of malignancy, (2) tumor marker concentrations exceeding reference limits (except CYFRA 21-1), and (3) having abnormal findings on a chest computed tomography scan.

After excluding 506 subjects, data of 4,098 apparently healthy subjects without malignancy or lung diseases were analyzed anonymously. Serum CYFRA 21-1 concentrations were measured using the Elecsys CYFRA 21-1 assay (Roche Diagnostics GmbH, Mannheim, Germany) on a cobas e 801 module immunology analyzer (Roche Diagnostics GmbH), which is based on an electrochemiluminescence immunoassay. After removing two outliers using the Dixon–Reed test, an RI was determined using the data of 4,096 subjects (median age, 45 years; range, 20–87 years). We applied the non-parametric method (2.5th and 97.5th percentiles) according to CLSI guidelines EP28-A3c, because the CYFRA 21-1 concentration showed a right-skewed distribution and did not follow a normal distribution according to the Kolmogorov–Smirnov test (Fig. 1) [14, 15]. The 90% confidence interval (CI) for each reference limit was calculated non-parametrically.

The subjects were divided into two sex groups (men, N=2,180; women, N=1,916) and four age groups (20–40 years, N=1,228; 41–50 years, N=1,840; 51–60 years, N=850; >60 years, N=178). The 20–30 years (N=124) and 31–40 years (N=1,104) groups were combined into a 20–40-years group because there was no significant difference in their median CYFRA 21-1 concentrations. The median CYFRA 21-1 concentration was compared between sex groups using the Mann–Whitney U test and between age groups using the Kruskal–Wallis test, with $P<0.05$ considered statistically significant. The statistical significance of the difference between subclass means was calculated as the statistic z-value by the standard normal deviate test to determine whether to partition RIs by sex [14]. The statistic z-value was compared with the critical value z* of 12.39 calculated as $3\times(\text{total number of samples}/240)^{1/2}$. If the statistic z-value exceeded z*, partitioning RIs by sex was recommended [14]. Statistical analyses were conducted using MedCalc Statistical Software (version 20.106; MedCalc Software, Ostend, Belgium).

For the 4,096 subjects, the RI of CYFRA 21-1 was 0.66–3.84 ng/mL (Table 1). The upper (97.5th percentile) reference limit in our study was higher than that reported in another study in Korean adults aged 20–60 years (3.59 ng/mL) [12]. This may be because our study included adults older than those in the previous study. When a single reference limit was established as the 95th percentile, it was the same as the value recommended by the manufacturer (3.3 ng/mL).

The sex- and age-specific RIs of CYFRA 21-1 are shown in Table 1. Although the median CYFRA 21-1 concentration was significantly higher in men than in women (1.56 vs. 1.41 ng/mL, $P<0.01$), the statistic z-value was 6.47, which did not exceed the critical value z* of 12.39. These results indicate that partitioning the RIs of CYFRA 21-1 by sex is not recommended. Regardless of sex, the median CYFRA 21-1 concentration showed

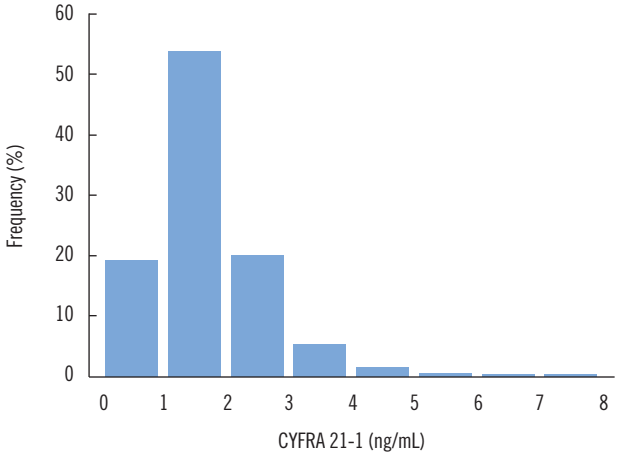

Fig. 1. Distribution of CYFRA 21-1 concentration in all subjects (N=4,096).

Table 1. Reference intervals of CYFRA 21-1 according to sex and age

Subjects	N (%)	Median value (ng/mL)	Lower reference limit* (90% CI, ng/mL)	Upper reference limit* (90% CI, ng/mL)
Total	4,096 (100.0)	1.49	0.66 (0.63–0.67)	3.84 (3.71–4.02)
Sex				
Men	2,180 (53.2)	1.56	0.68 (0.67–0.70)	4.09 (3.83–4.28)
Women	1,916 (46.8)	1.41	0.60 (0.58–0.64)	3.62 (3.46–3.81)
Age (yr)				
20–40	1,228 (30.0)	1.32	0.62 (0.56–0.65)	3.28 (3.17–3.41)
41–50	1,840 (44.9)	1.46	0.64 (0.60–0.68)	3.72 (3.58–3.85)
51–60	850 (20.8)	1.71	0.74 (0.68–0.81)	4.36 (4.02–4.74)
>60	178 (4.3)	2.02	0.96 (0.74–1.01)	5.00 (4.47–6.13)

*Lower and upper reference limits are the 2.5th and 97.5th percentiles, respectively.
Abbreviations: CI, confidence interval; N, number.

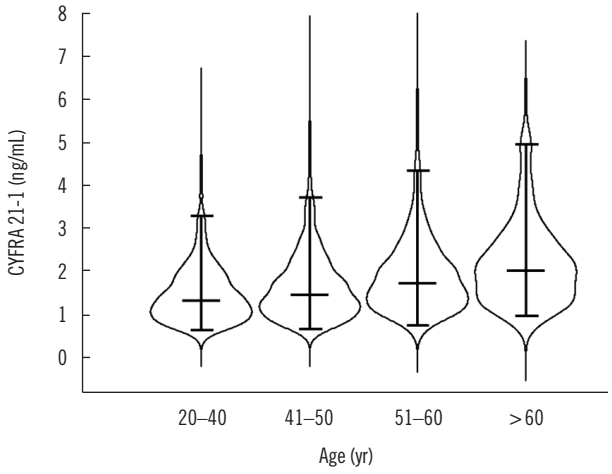

Fig. 2. Distribution of CYFRA 21-1 concentration according to age (N=4,096). The median CYFRA 21-1 concentration indicates significant differences between groups, determined using the Kruskal–Wallis test ($P<0.01$).

significant differences among all four age groups ($P<0.01$) (Fig. 2). In men and women, the difference in the median CYFRA 21-1 concentration by age was similar to the overall results. The CYFRA 21-1 concentration increased with age. Moreover, the upper reference limit was significantly higher, up to 5.00 ng/mL, in the group with subjects older than 60 years. The previous Korean study reported that the RI of CYFRA 21-1 showed a significant increase as of the age of 50 years [12]. Our results suggest that age-dependent RIs of CYFRA 21-1 should be applied, regardless of sex. CYFRA 21-1—an indicator of epithelial cell differentiation—increases with age because of the age-dependent alterations in epithelial cells [5, 16]. In line with our results, the serum CYFRA 21-1 concentration tends to increase with age in healthy Chinese adults [17]. CYFRA 21-1 concentrations higher than the upper reference limit have been associated with unfavorable prognosis [4, 8, 9]. Our results imply that age-dependent RIs, regardless of sex, should be applied to more effectively monitor the prognosis of lung cancer.

This study was limited in that it was conducted in apparently healthy subjects who visited our institution for a general health examination and were assessed only by a medical chart review. Since the information available in the medical charts of the subjects was limited, the RI was established without considering factors such as smoking, drug use, and underlying health conditions. Some studies have reported that smoking does not affect the CYFRA 21-1 concentration [18, 19]. However, another study has reported a significant difference in the CYFRA 21-1 concentration depending on smoking status [20]. Further studies are needed to evaluate the influence of factors such as smoking and underlying health conditions on the RI of CYFRA 21-1. Another limitation is that we established RIs of CYFRA 21-1 in healthy subjects and did not evaluate them in subjects with lung cancer. It is necessary to evaluate whether the RIs established in this study are clinically useful. As the RI of tumor markers cannot be an absolute criterion for diagnosing and monitoring cancer, comprehensive judgment, including clinical correlation, is required.

In conclusion, this is the first study to establish an RI of CYFRA 21-1 in Korean adults, including those older than 60 yrs, and the RI can be applied to both men and women. We also found that the CYFRA 21-1 concentration increased with age, regardless of sex, suggesting that age-dependent RIs of CYFRA 21-1 should be applied. Rather than using a single RI provided by the manufacturer, the RI of CYFRA 21-1 should be continually verified and established in each clinical laboratory.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Yoon S conceived the study, analyzed and interpreted the data, and wrote the draft. Lim YK collected and analyzed the data. Kweon OJ conceived the study and finalized the draft. Kim HR and Lee MK discussed the data and reviewed the manuscript. All authors have accepted responsibility for the entire content of the submitted manuscript and have approved submission.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

RESEARCH FUNDING

This study was supported by a National Research Foundation of Korea grant funded by the Korean government (grant No. 2020-R1A5A1018052).

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https://doi.org/10.3343/alm.2023.43.1.82

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