Reference Intervals of Alpha-Fetoprotein and Carcinoembryonic Antigen in the Apparently Healthy Population

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Background: The aim of this study was to calculate 95% reference intervals and double-sided limits of serum alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) according to the CLSI EP28-A3 guideline.

Material/Methods: Serum AFP and CEA values were measured in samples from 26,000 healthy subjects in the Shuyang area receiving general health checkups. The 95% reference intervals and upper limits were calculated by using MedCalc.

Results: We provided continuous reference intervals from 20 years old to 90 years old for AFP and CEA. The reference intervals were: AFP, 1.31–7.89 ng/ml (males) and 1.01–7.10 ng/ml (females); CEA, 0.51–4.86 ng/ml (males) and 0.35–3.45 ng/ml (females). AFP and CEA were significantly positively correlated with age in both males (r=0.196 and r=0.198) and females (r=0.121 and r=0.197).

Conclusions: Different races or populations and different detection systems may result in different reference intervals for AFP and CEA. Continuous reference intervals of age changes are more accurate than age groups.

MeSH Keywords: alpha-Fetoproteins • Carcinoembryonic Antigen • Reference Values

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Background

Serum alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) are the most widely used tumor markers in tumor treatment and post-treatment monitoring [1]. Reference intervals of CEA and AFP vary by ethnicity, living standard, geographical location, detection system, and many other factors. However, at present, most laboratories use the AFP and CEA reference intervals provided by the reagent manual. According to Chinese laws and regulations and industry standards, clinical laboratories must set reference intervals suitable for the region to meet the needs of clinical diagnosis and treatment. In this paper, according to CLSI EP28-A3, we established particular reference intervals based on a large sample of local healthy people.

Material and Methods

From April 2013 to May 2016, 26,000 healthy subjects were chosen from the Shuyang area health examination findings, focusing on the exclusion of liver and gastrointestinal diseases (such as positive HBV surface-antigen and data from questionnaires). Specimens were fasting venous blood. AFP and CEA were measured by electrochemiluminescence immunoassay on Beckman DXI system 800 immunoassay analyzers.

Figure 1. Histograms of alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) in subjects.
Table 1. Reference Intervals of AFP and CEA for apparently healthy population.

| Gender group | N  | Med | Lower limit (90% CL) | Upper limit (90% CL) | N  | Med | Lower limit (90% CL) | Upper limit (90% CL) |
|--------------|----|-----|----------------------|----------------------|----|-----|----------------------|----------------------|
| Male         | 16057 | 3.21 | 1.31 (1.29–1.33)     | 7.89 (7.79–8.00)     | 16775 | 1.64 | 0.51 (0.50–0.52)     | 4.86 (4.79–4.94)     |
| Female       | 8717  | 2.58 | 1.01 (0.99–1.03)     | 7.10 (6.90–7.27)     | 9095  | 1.10 | 0.35 (0.34–0.36)     | 3.45 (3.38–3.54)     |
| Total        | 24774 | 2.98 | 1.15 (1.13–1.17)     | 7.68 (7.60–7.79)     | 25870 | 1.43 | 0.42 (0.42–0.43)     | 4.53 (4.47–4.61)     |

95% Reference interval, Double-sided. Non-parametric percentile method (CLSI EP28-A3).

Figure 2. Scatter plots for alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA).
Figure 3. Continuous reference intervals for alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) concentration. The blue line represents the 50th percentile and the lower and upper red lines represent the 2.5th and 97.5th percentiles. The unit of AFP is ng/ml; the unit of CEA is ng/ml.

(Beckman Co., Ltd., USA). The detection limits of the assays are 0.1–1000 ng/ml (CEA) and 0.5–3000 ng/ml (AFP). Regular internal quality control (IQC) procedures and external quality assessment scheme (EQAS) were performed to validate the quality of AFP and CEA results. The Ethics Committee of Shuyang People’s Hospital approved this study. Reference samples were selected according to a previous report [2].

**Statistical analysis**

AFP and CEA data were pooled and showed by the non-parametric method using 95% (double-sided) reference intervals according to CLSI EP28-A3 guidelines [3]. Gaussian distribution of all data was tested using the D’Agostino-Pearson test. For outlier exclusion, Tukey’s outlier method was used. The ratio D/R proposed by Dixon was used to detect outliers, but we decided not to exclude any data unless clear analytical or biological reasons could be demonstrated, and all data analyses...
Table 2. Reference Intervals of AFP and CEA for the different group.

| Group   | N (Male) | Median (Male) | Lower limit (90% CI) | Upper limit (90% CI) | N (Female) | Median (Female) | Lower limit (90% CI) | Upper limit (90% CI) |
|---------|----------|---------------|----------------------|----------------------|------------|-----------------|----------------------|----------------------|
| Male    | 11690    | 3.09          | 1.25 (1.22–1.28)     | 7.74 (7.64–7.88)     | 12151      | 1.56            | 0.48 (0.47–0.49)     | 4.61 (4.51–4.71)     |
| 20% age <50 | 4317    | 3.53          | 1.57 (1.50–1.61)     | 8.35 (8.11–8.60)     | 4622       | 1.88            | 0.60 (0.58–0.61)     | 5.43 (5.23–5.69)     |
| 50% age |          |               |                      |                      |            |                 |                      |                      |
| Female  | 7345     | 2.5           | 0.99 (0.97–1.01)     | 6.82 (6.64–7.05)     | 7610       | 1.05            | 0.34 (0.33–0.35)     | 3.29 (3.18–3.40)     |
| 20% age <50 | 1422    | 3.02          | 1.30 (1.21–1.35)     | 8.13 (7.53–8.57)     | 1487       | 1.44            | 0.43 (0.42–0.48)     | 4.26 (3.90–4.48)     |
| 50% age |          |               |                      |                      |            |                 |                      |                      |

95% Reference interval, Double-sided. Non-parametric percentile method (CLSI EP28-A3).

Discussion

In this study, we analyzed serum AFP and CEA in a large cohort of apparently healthy individuals and established their reference intervals in Shuyang, Jiangsu Province, China. We found that AFP and CEA were positively correlated with age.

Therefore, reference intervals of different ages and sexes should be established. A study of AFP and CEA in the Fang Chenggang area [5] concluded that the reference interval of the instrument should be established. In the modern era of individualized medical care doctors assess the patient’s current test results, and the patient’s baseline value or the past test report are particularly important. If lacking basic values or previous data, inspection reports should be marked with the reference interval and detection system, especially in developing countries in regions such as Latin America, Asia, and Africa, where laboratory information systems are not common.

In this study, we found that AFP and CEA were positively correlated with age (Figure 2), showing that AFP and CEA tend to increase with age [6–8]. As shown in Figure 3, it is more reasonable to establish a different reference ranges for people over and under 50 years of age. In our study, the upper limits of the reference intervals are different from those of a previous report [4], possibly because the detection systems are different.

There are several limitations to this study. First, this was a single-center study using a single analysis system. Second, the subjects in this study were apparently healthy individuals. Third, smoking was not considered.

Conclusions

Different ethnicities and different detection systems may result in different reference intervals for AFP and CEA. In addition to providing the baseline, it is important to note the variations between the different detection systems and to establish the continuous reference intervals by age.

Declaration of interest

The authors declare no conflict of interest.
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