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Serum ARCHITECT PIVKA-II reference interval in healthy Chinese adults: Sub-analysis from a prospective multicenter study

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ABSTRACT

Background: Protein induced by vitamin K absence or antagonist-II (PIVKA-II) has been widely used as a biomarker for liver cancer diagnosis in Japan for decades. However, the reference intervals for serum ARCHITECT PIVKA-II have not been established in the Chinese population. Thus, this study aimed to measure serum PIVKA-II levels in healthy Chinese subjects.

Methods: This is a sub-analysis from the prospective, cross-sectional and multicenter study (ClinicalTrials.gov Identifier: NCT03047603). A total of 892 healthy participants (777 Han and 115 Uygur) with complete health checkup results were recruited from 7 regional centers in China. Serum PIVKA-II level was measured by ARCHITECT immunoassay. All 95% reference ranges were estimated by nonparametric method.

Results: The distribution of PIVKA-II values showed significant difference with ethnicity and sex, but not age. The 95% reference range of PIVKA-II was 13.62–40.38 mAU/ml in Han Chinese subjects and 15.16–53.74 mAU/ml in Uygur subjects. PIVKA-II level was significantly higher in males than in females ($P < 0.001$). The 95% reference range of PIVKA-II was 15.39–42.01 mAU/ml in Han males while 11.96–39.13 mAU/ml in Han females.

Conclusions: The reference interval of serum PIVKA-II on the Architect platform was established in healthy Chinese adults. This will be valuable for future clinical and laboratory studies performed using the Architect analyzer. Different ethnic backgrounds and analytical methods underline the need for redefining the reference interval of analytes such as PIVKA-II, in central laboratories in different countries.

1. Introduction

Protein induced by vitamin K absence or antagonist-II (PIVKA-II) is an abnormal form of prothrombin, which is also known as des-γ-carboxyprothrombin or acarboxy prothrombin [1–4]. PIVKA-II was first discovered in vitamin K deficient patients as implied in its name. It has

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been subsequently shown to have clinical potential in the screening for hepatocellular carcinoma (HCC). The sensitivity and specificity of PIVKA-II are superior to alpha-fetoprotein (AFP) [1–4]. It has gained increasing attention in HCC diagnosis. It is generally recommended to use PIVKA-II in combination with AFP.

Reference intervals (RIs) are health-associated benchmarks that enable clinicians to confidently interpret test results as normal or abnormal, and therefore critical for accurately diagnosing diseases [5–7]. RIs are essential for making clinical diagnosis, therapeutic management decisions, or other physiological assessment. RIs are supposed to be established or verified for each analyte and specimen source in every country or even in very clinical laboratory to achieve precise clinical assessment [8,9]. It is also important for the laboratory to carefully establish RIs by the laboratory according to standard protocols [10,11], for example, the clinical and laboratory standards institute (CLSI) guidelines.

PIVKA-II has been used in clinical practice for decades, but most of the clinical PIVKA-II experience is from Japan. However, according to the package insert, the 95% reference range showed significant ethnic differences between Japan ($n = 193$) and Europe (EU) ($n = 435$) [12,13]. Specimens were collected from 193 apparently healthy individuals in Japan and 435 apparently healthy individuals in EU. Serum PIVKA-II was measured using ARCHITECT PIVKA-II assay in accordance with CLSI Document C28-A3c. The 95% reference range was 11.12–32.01 mAU/mL in Japanese and 17.36–50.90 mAU/mL in Europeans. Such a difference between geographic regions highlights the need to establish reference intervals for different ethnic populations.

To further establish the clinical strategy for the use of PIVKA-II in other Asian countries, we initiated a prospective, cross-sectional, multicenter study of Han Chinese (ClinicalTrials.gov identifier: NCT03047603). In addition, we will compare the Han data with a group of Chinese known to have European ancestry - the Uygurs in Urumqi from the Xinjiang region. This report is a sub-analysis for the serum ARCHITECT PIVKA-II reference interval in healthy Chinese adults. Currently, no data is available for the RI of ARCHITECT PIVKA-II level in Chinese populations. Thus, these measurements will enable the determination of the 'normal range' of serum PIVKA-II in the people of China and other Asian countries.

2. Materials and methods

2.1. Study design and participants

This is a sub-analysis from the prospective, cross-sectional, multicenter study (ClinicalTrials.gov identifier: NCT03047603). The RI data in this sub-analysis were from seven hospitals (Peking University First Hospital, Renji Hospital of Shanghai Jiaotong University, Sichuan Provincial People's Hospital, Tongji Hospital affiliated to Huazhong University of Science and Technology, the First Hospital of Xi'an Jiaotong University, Gansu Provincial People's Hospital, and Xinjiang First Affiliated Hospital, Xinjiang Medical University) from April 2016 to June 2017 in China. The health status of patients were obtained from an overall health checkup, including physical examination and clinical laboratory tests, such as fasting plasma glucose, lipids levels, liver function tests, kidney function tests, and urinalysis.

The exclusion criteria were: 1) Age $<$ 18 years; 2) ALT $\geq$ 40 U/L or AST $\geq$ 40 U/L; 3) HBsAg(+); 4) History of hepatitis or cirrhosis; 5) History of cancer; 6) Diabetes mellitus or serum glucose level $>$ 6.1 mmol/L; 7) Pregnancy; 8) History of intravenous drug use; 9) Participation in another clinical study. This study was approved by the local ethics committee.

2.2. Measurement of serum PIVKA-II

Sufficient venous blood sample was taken from each participant at time of screening to obtain at least 1.2 mL serum. The serum sample was transferred equally into two separate tubes (600 μL each) and stored at −70 °C for later assay. Serum samples were ineligible for analysis if they were contaminated with fibrin, red blood cells or other particulate matter. Lipemic, hemolytic or icteric samples were also discarded.

ARCHITECT PIVKA-II immunoassay was used for measurement of PIVKA-II in serum samples in accordance with the defined protocol (Abbott Diagnostics, Abbott Park, IL).

The samples from Peking University First Hospital, Renji Hospital of Shanghai Jiaotong University and the First Hospital of Xi'an Jiaotong University were tested centrally at Peking University First Hospital. Other samples were tested at the respective study site. The limit of assay imprecision (coefficient of variance - CV) was set at ≤10%.

2.3. Statistical analysis

Reference intervals were derived according to the CLSI C28-A3 guideline [10]. The distribution of PIVKA-II data was tested for normality by Shapiro-Wilk method. If non-Gaussian, reference intervals and statistical significance would be estimated by nonparametric method. The D/R ratio proposed by Dixon was used to identify outliers [14]. Reference values were further stratified by sex, age group (18–29, 30–49, 50–69, and ≥70 years of age), and ethnicity. Statistical analysis was conducted by using MedCalc Statistical Software version 15.2.2 (MedCalc Software, Ostend, Belgium) and SPSS. All tests were two-sided and the significance level was set as 0.05.

3. Results

3.1. Demographic and clinical characteristics of study participants

A total of 892 participants (mean age of 41.6 ± 12.6 years; 455 males) from 7 representative regional centers in China were finally included in this analysis. The demographic characteristics of the study participants are presented in Table 1.

Table 1
Demographic characteristics of the study participants.

Parameter	Beijing ($n = 120$)	Shanghai ($n = 110$)	Chengdu ($n = 120$)	Wuhan ($n = 100$)	Xi'an ($n = 77$)	Lanzhou ($n = 135$)	Urumqi (Han) ($n = 115$)	Urumqi (Uygur) ($n = 115$)	Total ($N = 892$)
Sex									
Male	73 (60.8)	63 (57.3)	53 (44.2)	30 (30.0)	31 (40.3)	64 (47.4)	82 (71.3)	59 (51.3)	455 (51.0)
Female	47 (39.2)	47 (42.7)	67 (55.8)	70 (70.0)	46 (59.7)	71 (52.6)	33 (28.7)	56 (48.7)	437 (49.0)
Age, years	39.5 ± 13.9	42.0 ± 11.2	42.0 ± 10.9	36.6 ± 11.2	40.8 ± 13.6	40.7 ± 11.1	43.0 ± 12.1	47.2 ± 14.4	41.6 ± 12.6
18–29	39 (32.5)	18 (16.4)	20 (16.7)	39 (39.0)	17 (22.1)	18 (13.3)	15 (13.0)	8 (7.0)	174 (19.5)
30–49	52 (43.3)	59 (53.6)	71 (59.1)	43 (43.0)	48 (62.3)	85 (63.0)	66 (57.4)	62 (53.9)	485 (54.4)
50–69	24 (20.0)	32 (29.1)	29 (24.2)	17 (17.0)	9 (11.7)	30 (22.2)	30 (26.1)	35 (30.4)	206 (23.1)
≥70	5 (4.2)	1 (0.9)	0 (0)	1 (1.0)	3 (3.9)	2 (1.5)	4 (3.5)	10 (8.7)	27 (3.0)

Data are presented as n (%) or mean ± standard deviation.