

## Original Article

# Establishing reference intervals of gastrin-releasing peptide precursor by analysis of laboratory-stored data

Li-Yue Zhao<sup>1\*</sup>, Yang-Chun Feng<sup>1\*</sup>, Zhao-Xia Zhang<sup>2</sup>, Yan-Chun Huang<sup>1</sup>

<sup>1</sup>Clinical Laboratory Center, The Tumor Hospital Affiliated to Xin Jiang Medical University, Urumqi 830011, Xinjiang, China; <sup>2</sup>Clinical Laboratory Center, The First Affiliated Hospital of Xin Jiang Medical University, Urumqi 830011, Xinjiang, China. \*Equal contributors.

Received March 15, 2017; Accepted January 28, 2018; Epub April 15, 2018; Published April 30, 2018

**Abstract:** Objective: To establish and verify the feasibility of reference intervals (RIs) of gastrin-releasing peptide precursor (ProGRP) by analysis of laboratory-stored data. Methods: The detection results of ProGRP from outpatients and health examinations were collected from the population in the First Hospital and Tumor Hospital Affiliated to Xinjiang Medical University from January 2015 to December 2016. Simultaneously, the general characteristics of the population were collected using a laboratory information management system. A total of 16384 ProGRP cases were collected. All ProGRP tests were measured using Abbott fully automated microparticle luminescence analyzer. The ProGRP RIs of different sexes and ages were established by differentially optimized statistical methods, which included the Hoffmann method, the NLT Hoffmann method (in which the natural logarithm was calculated first and then the Hoffmann method was performed), the Tukey method, the NLT Tukey method (in which the natural logarithm was calculated first and then the Tukey method was performed), and the non-parametric method. Results: There were no significant differences in the ProGRP results between males and females ( $P > 0.05$ ). The ages were positively correlated with the measured value of the ProGRP ( $R = 0.342$ ,  $P < 0.05$ ), which could be grouped according to ages. The established RIs of ProGRP in this study was 8.53-67.575 pg/ml. Conclusion: According to different statistical methods, we established the RIs of ProGRP in this study, which will be of certain reference value to the future laboratory studies.

**Keywords:** Gastrin-releasing peptide precursor, reference intervals

## Introduction

Gastrin releasing peptide (GRP) is a neuropeptide substance similar to bombesin, which is composed of 27 amino acids, but it is extremely unstable [1]. The ProGRP fragment (31-98) obtained from the translation product GRP is currently used for detection [2]. Studies have shown that the ProGRP fragment (31-98) in serum of patients with SCLC was a stable marker [3].

The ProGRP RIs recommended by the current reagent manufacturers is 0-35 pg/ml. The investigators tested samples of 194 healthy individuals who had no lung disease and nephropathy. Including 170 males and 24 females, aged 20-63 years, with an average age of 45.7 years. The results showed that the ProGRP value of 95% samples was less than or equal to 35 pg/ml. However, in our clinical

work, we found that the detection values of many physical examinations were higher than this range. This phenomenon has brought confusion to clinicians and increased psychological pressure on the patients. Therefore, it is necessary to re-establish the RIs of the index.

Currently, there are two ways to establish RIs. The internationally recommended method is direct method. However, this method is complex and consumes significant manpower and material resources. Therefore, the indirect method came into being: through the use of large amounts of data stored in laboratory management systems, RIs can be based on statistical methods established and popularized by the majority of scholars [4-7]. At the same time, our laboratory also used large data to establish the RIs of TSH [8], so as to increase our credibility of this study.

# Establishing reference intervals of ProGRP

## Materials and methods

### *Source of sample materials*

The general characteristics data used in this study was obtained from the Laboratory Information Management System of the First and Third Affiliated Hospital of Xin Jiang Medical University. A total of 16384 ProGRP cases were collected. Taking into account that physical examinations were usually a person older than 18-year-olds, therefore, this study only analyzed the indicators of adults over 18 years of age. First, make clear the inclusion and exclusion criteria in this study. The inclusion criteria included the following: complete and accurate laboratory tests, Physical health, physical examination and outpatients, no previous kidney disease [9], no history of lung disease. The exclusion criteria included the following: results after the first of multiple tests following the same treatment, incomplete personal information of the patient, or other chronic diseases. The final data included 12380 cases, 7943 males and 4437 females.

### *Instruments and reagents*

This study was used the American Abbott i2000 automatic microparticle chemiluminescence immunoassay analyzer and Abbott support of the ProGRP detection kits and standards [10]. Internal quality control was performed using Bio-Rad's conventional Tumor Marker Item Control. Daily internal quality control was performed using the Westgard Multiple IQC rules, etc. The cumulative coefficient of variation in this lab was 5%.

### *Research methods*

#### Normality test of data

The data was analyzed and normalized by the skewness-kurtosis (s-k) value method. When the s-k values of the test were less than 1.96 times of the standard deviation, this distribution was considered a normal distribution [11]. For non-normal distributions, the logarithmic data could convert to a normal distribution curve. By plotting the histogram of frequency distribution, the situation of normal distribution was presented visually.

#### Eliminating outliers

According to the clinical laboratory standard document EP28-A3, the Dixon method is the

recommended method for eliminating outliers. The operation steps are as follows. First, we calculated the range R (the difference between maximum and minimum) and then selected three consecutive measurements, A1, A2 and A3, and performed the following calculation:  $X1 = |(A2-A1)|/R$ ,  $X2 = |(A3-A2)|/R$ . If  $X1/R > 1/3$  or  $X2/R > 1/3$ , then the value was removed as an outlier [12]. The loop was screened until all outliers were removed. According to this method, the final data included 11274 cases, of which there were 7178 male cases and 4096 female cases.

#### Data analysis

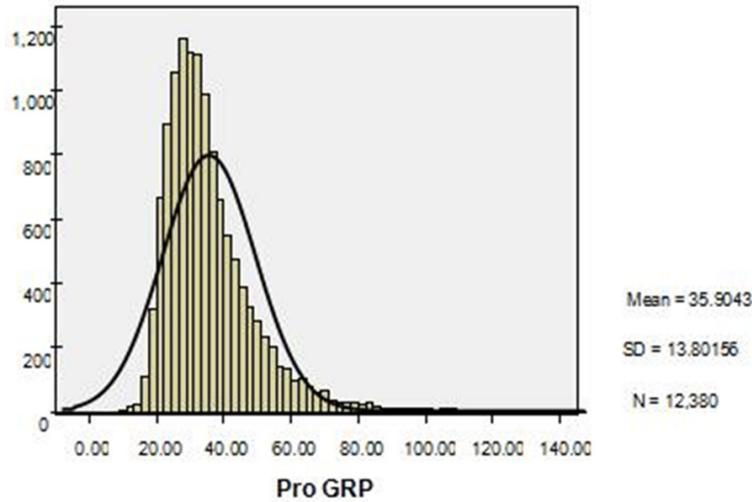
The final data included 11274 cases, including 7178 males and 4096 females. The age breakdown was as follows: 18-30 years old in 257 cases, 31-50 years old in 3347 cases, 51-70 years old in 5200 cases, and more than 70 years old in 2470 cases. The data was grouped according to sexes, and the non-parametric rank-sum test was used to test whether there were significant differences between the sexes. If there were significant differences, patients were grouped according to gender. Spearman correlation analysis was used to determine whether the measured value was age-related. If there was a correlation, the RIs was established according to age ([Supplementary Data](#)).

#### Five methods used to establish the RIs

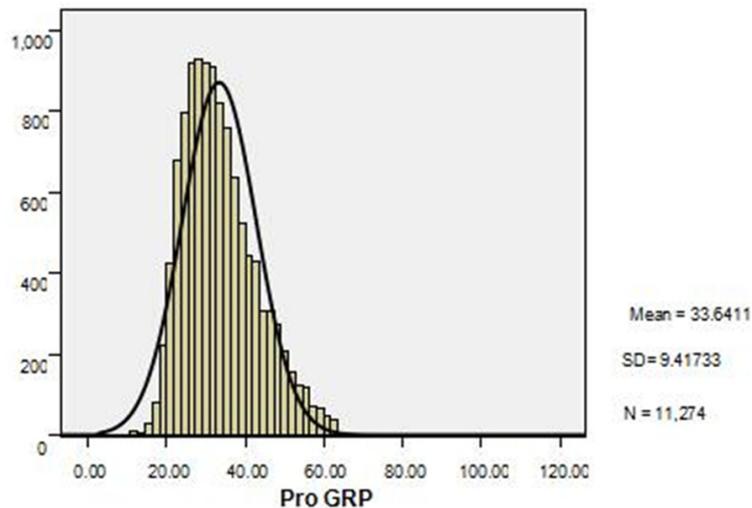
*The Hoffmann analysis method:* The Hoffmann method was a cumulative frequency probability map that was presented by Hoffmann et al in 1963 and constructed by observing and selecting the linear part of the data [13]. According to the literature, the criterion of selecting the linear part of the data was to set a linear deviation of 10% as an acceptable error standard [14]. Using ProGRP measurements as the dependent variable Y and the cumulative probability for the independent variable X, fitting the linear regression equation was as follows:  $Y_i = \alpha + \beta X_i + e_i$ . Therefore,  $X = 2.5\%$ ,  $X = 97.5\%$ ,  $RI_{\min} = \alpha + 0.025\beta$  and  $RI_{\max} = \alpha + 0.975\beta$ .  $RI_{\min}$  and  $RI_{\max}$  were the lower and upper limits of the 95% reference interval, respectively [15].

*The NLT Hoffmann method:* Herein, the Hoffmann method was applied after taking the natural logarithm of the data. The core of this step was to eliminate outliers too.

## Establishing reference intervals of ProGRP



**Figure 1.** Histogram of 12380 ProGRP cases: horizontal ordinates represent ProGRP test values, and vertical ordinates represent the frequency. The curve is the ProGRP normal distribution curve.



**Figure 2.** Histogram of 11274 ProGRP cases: horizontal ordinates represent ProGRP test values, and vertical ordinates represent the frequency. The curve is the ProGRP normal distribution curve.

*The Tukey analysis method:* American statistician John Tukey put forward the Tukey method in 1977. The Tukey method calculated the interquartile range (IQR) as the difference between the 25th and 75th percentiles. All values below the 25th percentile minus 1.5 times the IQR were removed, and at the same time, all values above the 75th percentile plus 1.5 times the IQR were removed [16].

*NLT Tukey method:* The Tukey method was applied herein after calculating the natural logarithm of the data.

*Non-parametric statistical method:* After ranking the final data, the non-parametric statistical method was used to calculate the values of 2.5% and 97.5%, respectively.

### Statistical analysis

Microsoft Office Excel 2007 and SPSS19.0 software were used for analysis.

Based on biological variability ( $RCV = 2^{1/2} \times Z \times [CVA^2 + CVI^2]^{1/2}$ ), we calculated whether the results had significant meaning and compared them with RIs currently used in the lab.

We then used the “1/3” rule of the EP28-A3 document to determine whether the RIs in this study could be accepted [17].

Compared the RIs used at present with the RIs established in this experiment.

## Results

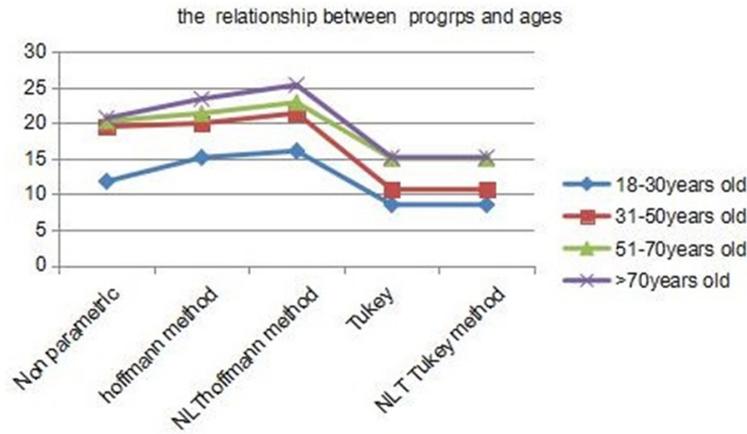
### *Data distribution*

Following selection, 12380 cases were included in this study. Plotting the frequency distribution histogram showed that after converting the data, the data exhibited approximately normal distribution which was shown in **Figure 1**.

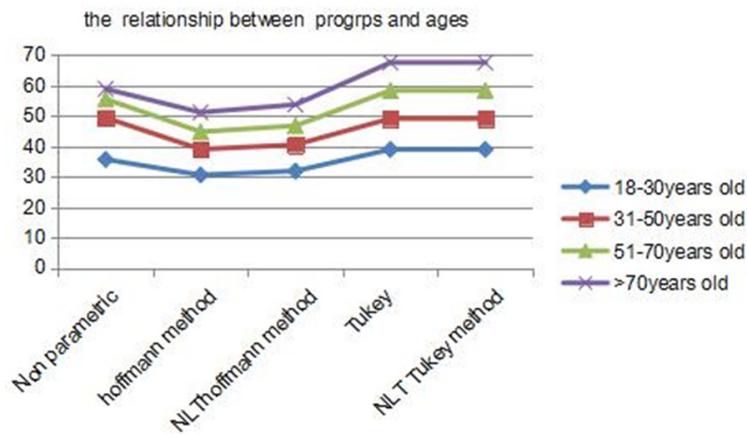
### *Eliminating outliers*

By means of the Dixon method, the final data included 11274 cases, of which there were 7178 males and 4096 females. The age breakdown was as follows: 18-30 years old in 257 cases, 31-50 years old in 3347 cases, 51-70 years old in 5200 cases, and more than 70 years old in 2470 cases. We used this information to draw the frequency distribution histogram and observe the distribution of the data in **Figure 2**.

## Establishing reference intervals of ProGRP



**Figure 3.** Line charts showing lower limits in the RIs of different ages using different statistical methods.



**Figure 4.** Line charts showing upper limits in the RIs of different ages using different statistical methods.

### Detection results of ProGRP in different gender

The SPSS software was used to calculate the non-parametric rank sum test, and the results showed that there was no significant difference between males and females ( $P > 0.05$ ) and the RIs could not be set according to gender.

### Correlation between the results of ProGRP and ages

Using the Spearman correlation statistical analysis, the final data included 11274 cases. According to SPSS and Excel statistical analyses, the ProGRP test results and different ages have a positive correlation ( $r = 0.342$ ,  $P < 0.05$ ).

### Establishment of RIs for different statistical methods

RIs were obtained by using the Hoffmann method, the NLT Hoffmann method, the Tukey method, the NLT Tukey method and the non-parametric method. The results respectively were as follows: at the age of 18-30 years old, 15.159-30.578 pg/ml, 16.087-31.881 pg/ml, 8.53-38.973 pg/ml, 8.53-38.973 pg/ml, and 11.806-35.680 pg/ml; at the age of 31-50 years old, 19.984-39.041 pg/ml, 21.413-40.488 pg/ml, 10.663-49.123 pg/ml, 10.663-49.123 pg/ml, and 19.507-49.413 pg/ml; at the age of 51-70 years old, 21.379-44.777 pg/ml, 22.920-46.759 pg/ml, 15.02-58.385 pg/ml, 15.02-58.385 pg/ml, and 20.280-55.570 pg/ml; and at the age of more than 70 years old, 23.418-51.177 pg/ml, 25.381-53.732 pg/ml, 15.20-67.575 pg/ml, 15.20-67.575 pg/ml, and 20.705-58.903 pg/ml, which were shown in **Figures 3 and 4**.

The relative variability of Pro-GRP was 14.68% [18], and the cumulative coefficient of variation in this lab was 5%.

Using the formula  $RCV = 2^{1/2} \times Z \times [CVA^2 + CVI^2]^{1/2}$ , we were able to calculate whether the results of this study were significant. Z represented a 95% confidence interval, for which 1.96 was used. CVA represented the laboratory cumulative variation coefficient and CVI represented the individual biological variability. In this study,  $RCV = 2^{1/2} \times 1.96 \times [0.052 + 0.147]^{1/2} = 42.85\%$ . The RIs currently used in the laboratory is 0-50 pg/ml. Compared the differences in the RIs established by the different methods and different ages (including  $RCV_{min}$  and  $RCV_{max}$ ).

$RCV_{min} = (RI_{min} - RI'_{min}) / RI'_{min}$ ,  $RI'_{min}$  is the smaller of two.

$RCV_{max} = (RI_{max} - RI'_{max}) / RI'_{max}$ ,  $RI'_{max}$  is the larger of two.

## Establishing reference intervals of ProGRP

**Table 1.** Comparison of reference change values between RIs of different ages and RIs provided by manufacturer

Grouping	RCV (%)	Non- parametric	Hoffmann Method	NLT Hoffmann Method	Tukey Method
18-30 years old	RCV <sub>min</sub>	0	0	0	0
	RCV <sub>max</sub>	40.13	63.52 <sup>a</sup>	56.83 <sup>a</sup>	28.29
31-50 years old	RCV <sub>min</sub>	0	0	0	0
	RCV <sub>max</sub>	1.19	28.07	23.49	1.79
51-70 years old	RCV <sub>min</sub>	0	0	0	0
	RCV <sub>max</sub>	11.14	11.66	6.93	16.77
Above 70 years old	RCV <sub>min</sub>	0	0	0	0
	RCV <sub>max</sub>	17.81	1.18	7.46	35.15

<sup>a</sup>Either RCV<sub>min</sub> or RCV<sub>max</sub> calculated between two reference intervals is larger than the allowable RCV.

$$RCV_{min} = (11.806-0)/0 \times 100\% = 0\%$$

$$RCV_{max} = (50-35.68)/35.68 \times 100\% = 40.13\%$$

Compared the calculated RCV<sub>min</sub> and RCV<sub>max</sub> with RCV (42.85%). If RCV<sub>min</sub> and RCV<sub>max</sub> are smaller than RCV, there is no difference between two RIs; if RCV<sub>min</sub> and RCV<sub>max</sub> are larger than RCV, there is difference between two RIs. It could be seen from **Table 1**.

Based on the EP28-A3 document proposed by CLSI to calculate RIs, ProGRP was measured in 80 healthy subjects in different four groups, if the RIs established by different age groups did not exceed the threshold value in more than 3 cases, which could be seen from **Table 2** the RIs established by each group can be accepted. The RIs established in this study was validated in this laboratory, and the normal RIs of ProGRP was 8.53-67.575 pg/ml. The lowest value and the highest value are higher than the RIs currently used. From the **Table 3** we can see, the RIs established by this study is better able to cover healthy people.

### Discussion

Presently, the laboratory method used to establish the RIs is a direct method provided according to EP28-A3. However, it is very difficult to meet the standards in actual operations. Thus, many scholars have considered using an indirect method based on large sample statistical analyses such as the Robust method or Bhattacharya method to calculate RIs [19]. This study used five different statistical methods to establish the ProGRP RIs.

The Hoffmann method was used in this study. In 2010, The American Journal of Clinical Pathology, Katayev and his colleagues published an article with the RIs setting based on the method published by Hoffmann in 1963 [5]. In this article, they introduced how to make use of the data stored in multicenter laboratories to establish new statistical methods for the RIs of hemoglobin, creatini-

ne, urea nitrogen and thyrotropin [20]. Dorizzi repeated the RIs for TSH established by this method, and the results were consistent with the results of Katayev. Therefore, this method is recommended by many scholars, both domestic and overseas [21]. The Hoffmann method requires the data to obey the normal distribution, and if the data are not included in the normal distribution, this method may be biased. There are several means to transform data, such as logarithmic, Box-Cox conversion and others. After log transformation, the data is converted to approximate normal distribution conditions (Skewness = 0.7, Kurtosis = 0.3). The most important step for establishing RIs using the Hoffmann method is to select the appropriate method to eliminate abnormal values. The NLT Hoffmann method also need to eliminate abnormal values. The method chosen in this study is the Dixon method, which is recommended by CLSI. Then plot the cumulative probability distribution of the data, but the linear part selected in this step has a certain degree of subjectivity. Based on the cumulative probability distribution curve, visualization or subjective set deviation values of 5%, 10%, 15% or other ranges can be used to choose the appropriate deviation value. Now there is no scientific conclusion and selection method. Studies have shown that 10% deviation using the Hoffman method is the best choice because it may be more reasonable.

First using the natural logarithm and then using the Hoffmann method analysis essentially reduces the variance and the absolute value of the data. Additionally, it is convenient to cal-

## Establishing reference intervals of ProGRP

**Table 2.** Verify the RIs in different ages established in this study

Grouping	N	Reagent manufacturer (pg/ml)	Reference intervals (in this study)	Reference intervals validation
18-30 years old	20	0-50	8.53-38.973	8.90-33.610
31-50 years old	20	0-50	10.663-49.413	11.330-32.50
51-70 years old	20	0-50	15.020-58.385	15.74-49.120
Above 70 years old	20	0-50	15.2-67.575	16.930-51.60

**Table 3.** Change frequencies of normal and abnormal ProGRP with new RI and manufacturer RI

	Normal (%)	Abnormal (%)
Manufacturer RI	10536 (93.45)	738 (6.55)
Established RI	11272 (99.98)	2 (0.02)

culate. Taking the natural logarithm does not change the nature of the data or the correlation between the variables, but the compression scale makes the data more stable. From the results of this study, it can be seen that the RIs (0-51.177 pg/ml) established by Hoffmann method is the most similar to the RIs (0-50 pg/ml) used in the lab presently. This method can be used to obtain a large enough sample size to cover different sexes, ages, and clinical test results that are particularly applicable.

RIs were also established by using the Tukey method. The characteristics of the data can be clearly identified and the distribution characteristics (the minimum value, the next four digits, the median, the top four digits and the maximum value) can be displayed intuitively. In this study, the cartographic method was used to compute the RIs across different ages, but this method could not provide precise measurements of skewness or weight of the data distribution. For statistics using larger data sets, the information reflected by the shape is more ambiguous. There are some limitations in using the median to represent the overall average.

Next, we took the natural logarithm first, and then analyzed the data using the Tukey method. Since the RIs calculated by the two methods (Tukey method and NLT Tukey method) are the same, only one set of RIs using the Tukey method is shown in this study. This method is used less often in the literature and is often used as an outlier removal method. It has high

removal efficiency and accuracy. At the beginning of 2016, David, a scholar of Israel, published a reference value range of TSH, FT3 and FT4 based on the Hoffmann method and the Tukey method to compare the reference value range and obtain the appropriate RIs. However, its

scientific and practical value need to be further explored and studied.

The non-parametric method is a commonly used method to calculate biological RIs. The sample numbers have higher requirements, but the specific distribution of the data is no hard requirements. In this study, the RIs calculated using the non-parametric method are higher than the RIs calculated by other methods. Because this method is simple and the metering level is low, it is not as sensitive as the parameter statistical method.

Using the formula for RCV biological variation, we compared the RIs of ProGRP used currently and the RIs calculated using the different statistical methods in this study. After statistical analysis, there was no significant statistical difference between different statistical methods and the current data. Although there is a large quantity of data in this study, it does not exclude the existence of abnormal physical examination data that may be included. The statistical methods used (the Tukey method in particular) are not popular nor applied by the majority of scholars and need to be further explored. If there is a patient with a ProGRP value slightly higher than the upper limit of the RIs, whether or not the patient has a lung disease is still an issue to be discussed. The upper limits of the RIs depends on the unilateral 95th percentile. In fact, there are some physical examinees who are in good health, but the detection value of ProGRP is really higher than the upper limit of the RIs. Under these circumstances, the patient should be carefully analyzed to reduce the need for treatment or unnecessary examinations.

Over time, hospital information management systems have been gradually improved. The indirect method is simpler than the direct method, and the price and technical requirements

## Establishing reference intervals of ProGRP

are low. The method is especially suitable to establish RIs according to the indexes of laboratories in different regions and across different populations.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Yan-Chun Huang, Clinical Laboratory Center, The Tumor Hospital Affiliated to Xin Jiang Medical University, No.789 Suzhou Street, Urumqi 830011, Xinjiang, China. Tel: 0991-7819420; E-mail: 541509330@qq.com

### References

- [1] Stieber P, Dienemann H, Schalhorn A, Schmitt UM, Reinmiedl J, Hofmann K and Yamaguchi K. Pro-gastrin-releasing peptide (ProGRP)—a useful marker in small cell lung carcinomas. *Anticancer Res* 1999; 19: 2673-2678.
- [2] Aoyagi K, Miyake Y and Urakami K. Enzyme immunoassay of immunoreactive progastrin-releasing peptide (31-98) as tumor marker for small-cell lung carcinoma: development and evaluation. *Clin Chem* 1995; 41: 537-543.
- [3] Oh HJ, Park HY, Kim KH, Park CK, Shin HJ, Lim JH and Kwon YS. Progastrin-releasing peptide as a diagnostic and therapeutic biomarker of small cell lung cancer. *J Thorac Dis* 2016; 8: 2530-2537.
- [4] Aral H, Usta M, Cilingirturk AM, Inal BB, Bilgi PT and Guvenen G. Verifying reference intervals for coagulation tests by using stored data. *Scand J Clin Lab Invest* 2011; 71: 647-652.
- [5] Zhang GM, Guo XX, Zhu BL, Zhang GM, Bai SM, Wang HJ, Ma XB and Zhou YT. Establishing reference intervals of aspartate aminotransferase-to-platelet ratio index for apparently healthy elderly. *Clin Lab* 2016; 62: 135-140.
- [6] Henny J. Determining and verifying reference intervals in clinical laboratories. *Ann Biol Clin* 2011; 69: 229-237.
- [7] Shaw JL, Cohen A, Konforte D, Binesh-Marvasti T, Colantonio DA and Adeli K. Validity of establishing pediatric reference intervals based on hospital patient data: a comparison of the modified Hoffmann approach to CALIPER reference intervals obtained in healthy children. *Clin Biochem* 2014; 47: 166-172.
- [8] Feng Y, Bian W, Mu C, Xu Y, Wang F, Qiao W, Huang Y. Establish and verify TSH reference intervals using optimized statistical method by analyzing laboratory-stored data. *J Endocrinol Invest* 2014; 37: 277-284.
- [9] Molina R, Filella X and Auge JM. ProGRP: a new biomarker for small cell lung cancer. *Clin Biochem* 2004; 37: 505-511.
- [10] Stieber P, Molina R and Dowell B. Clinical evaluation of the ARCHITECT ProGRP assay. *Thoracic Oncology* 2008; 3: 236.
- [11] Zhou JX. Probability and mathematical statistics. China Statistics Press 2007; 232-239.
- [12] Aitkenhead H and Heales SJ. Establishment of paediatric age-related reference intervals for serum prolactin to aid in the diagnosis of neurometabolic conditions affecting dopamine metabolism. *Ann Clin Biochem* 2013; 50: 156-158.
- [13] Katayev A, Balciza C and Seccombe DW. Establishing reference intervals for clinical laboratory test results: is there a better way. *Am J Clin Pathol* 2010; 133: 180-186.
- [14] Katayev A, Fleming JK, Luo D, Fisher AH and Sharp TM. Reference intervals data mining: no longer a probability paper method. *Am J Clin Pathol* 2015; 143: 134-142.
- [15] Hoffmann RG. Statistics in the practice of medicine. *JAMA* 1963; 185: 864-873.
- [16] Strich D, Karavani G, Levin S, Edri S and Gillis D. Normal limits for serum thyrotropin vary greatly depending on method. *Clinical Endocrinology* 2016; 85: 110-115.
- [17] Gary L, Sousan A, James C, Ferruccio C, Paul H and Amadeo P. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline-third edition. *Clin Lab Standards Institute* 2010; 2: 1-59.
- [18] Qi Z, Zhang L, Chen Y, Ma X, Gao X, Du J, Zhang F, Cheng X and Cui W. Biological variations of seven tumor markers. *Clin Chim Acta* 2015; 450: 233-236.
- [19] Thygesen K, Alpert JS and White HD. Universal definition of myocardial infarction. *J Am Coll Cardiol* 2007; 50: 2173-2195.
- [20] Horowitz GL. Estimating reference intervals. *Am J Clin Pathol* 2010; 133: 175-177.
- [21] Dorizzi RM, Giannone G, Cambiaso P, Cappa M and Muraca M. Indirect methods for TSH reference interval: at last fit for purpose? *Am J Clin Pathol* 2011; 135: 167-174.