YANG SHENG, WU MOUTIAN, XIE MINHAO, DENG JING, YANG ZHIYONG, FANG ZILONG, GUO JIANJUN, ZHANG CHANGJIU:
Statistical Results of Routine Blood Test for Five Parameters Related to Sydney On-Model
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Abstract
Blood testing has been performed in China since the year 1998. In the earlier stage, only Haematocrit (Hct) has been tested with the UCI criteria as a health factor. After the On-model was published in the year 2000, most routine blood tests were based on combination of UCI criteria and the On-model. In this paper, results of blood tests for three years were statistically analyzed. Statistical distribution of the results of the On-model related parameters were reviewed and discussed. Non-parametric method was implemented to investigate the normal ranges of these measured blood parameters.

Preface
Although the International Olympic Committee (IOC) has banned the use of recombinant human erythropoietin (rhEPO) since 1989, the methods for detecting rhEPO were not formulated until in the year 2000 [1]. In the earlier stage, only Hct has been tested using UCI criteria for the athletic health. In 2000, with the co-operation of the Sydney IOC accredited laboratory, 24 trials were carried out in China, which together with studies in Australia allowed various models for blood EPO (such as the ON model) to be published. Since then, our routine blood tests were based on the combination of the UCI criteria and the ON-model. Results of blood tests for three years, 2000 with 141 cases, 2001 with 425 cases and 2002 with 228
cases, were statistically analyzed. The aim of this paper is focused on the reference ranges of the parameters for Chinese athletes.

Subjects
There were in total 794 Chinese national level athletes (369 males, 425 females) selected as the subjects for blood tests before sports competition in 3 years (including the 2000 Sydney Olympic Games, the 9th Chinese National Sports Games, the 2002 Busan Asian Sports Games). All the subjects were Chinese. They were selected by the testing section of the Chinese Olympic Committee Anti-Doping Commission (COCADC).

Blood Samples Collection and Measurement
Blood was drawn from the veins of the forearm by qualified medical staff in the morning before breakfast. Samples were drawn into one 8 mL serum separation Vacuette tube with clot activator (Greiner Labortecnik, Frikenhausen, Germany), and one 2 mL K$_3$EDTA Vacuette tube. In China, all the samples were shipped to our laboratory within 8 hours by doping control officers (DCOs). Whole blood samples were measured for Hct, Macro%, and RetHct using the Advia-120 analyzer (Bayer, USA). Soluble transferrin receptor (sTfR) was tested using an ELISA kit (R&D systems, USA). Serum EPO was measured using the Immulite Chemiluminescent instrument (DPC, USA).

Statistics
Independent sample t-test (2-tailed) was performed to evaluate the annual variance. Gaussian distribution was tested using Kolmogorov-Smirnov test as well as coefficients of skewness and kurtosis. Analyses were conducted using SPSS for Windows software with release version 10.0.1 (SPSS Inc., USA). The non-parametric method were used to develop the 95% reference ranges.[2]

Results and Statistical Tests
1. The results of female (Table 1), male (Table 2) and Total (Table 3) of Hct, Macro%,

356
RetHct, EPO and sTfR were given by means and standard deviations.

Table 1 Annual Female parameters expressed in Means and SDs.

<table>
<thead>
<tr>
<th>Year</th>
<th>Hct (L/L)</th>
<th>Macro%*</th>
<th>RetHct (L/L)</th>
<th>EPO (mIU/ml)</th>
<th>sTfR (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000, n=106, 105*</td>
<td>0.40±0.028*</td>
<td>2.25±1.61*</td>
<td>6.68x10^3±4.09x10^-3</td>
<td>9.15±4.11</td>
<td>18.53±5.31</td>
</tr>
<tr>
<td>2001, n=209</td>
<td>0.41±0.027*</td>
<td>1.05±1.03*</td>
<td>6.21x10^3±2.33x10^-3</td>
<td>9.16±5.35*</td>
<td>19.70±5.51*</td>
</tr>
<tr>
<td>2002, n=110</td>
<td>0.39±0.024*</td>
<td>0.75±1.17*</td>
<td>8.46x10^3±2.70x10^-3</td>
<td>10.49±4.05*</td>
<td>22.57±5.08*</td>
</tr>
</tbody>
</table>

Symbol * indicated that there was one data missing of Macro%; Symbol # represented p<0.01, using data of the year 2000 compared with the year 2001; Symbol $ represented p<0.05, using data of the year 2001 compared with the year 2002; Symbol $ represented p<0.05, using data of the year 2000 compared with the year 2002.

Table 2 Annual Male parameters expressed in Means and SDs.

<table>
<thead>
<tr>
<th>Year</th>
<th>Hct (L/L)</th>
<th>Macro%*</th>
<th>RetHct (L/L)</th>
<th>Epo (mIU/mL)</th>
<th>sTfR (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000, n=35</td>
<td>0.45±0.026</td>
<td>1.46±1.12*</td>
<td>6.27x10^3±1.85x10^-3</td>
<td>9.24±3.51</td>
<td>18.16±4.61</td>
</tr>
<tr>
<td>2001, n=216</td>
<td>0.44±0.027*</td>
<td>0.66±0.72</td>
<td>7.19x10^3±2.38x10^-3</td>
<td>8.87±3.37*</td>
<td>19.69±4.35*</td>
</tr>
<tr>
<td>2002, n=118</td>
<td>0.44±0.023</td>
<td>0.61±0.92*</td>
<td>8.64x10^3±2.69x10^-3</td>
<td>10.09±4.26</td>
<td>22.83±5.52*</td>
</tr>
</tbody>
</table>

Symbol * indicated that there was one data missing of Macro%; Symbol # represented p<0.01, Symbol @ represented p<0.05, using data of the year 2000 compared with the year 2001; Symbol $ represented p<0.05, using data of the year 2001 compared with the year 2002; Symbol $ represented p<0.05, using data of the year 2000 compared with the year 2002.

Table 3. Total Data of Female and Male parameters expressed in Means and SDs

<table>
<thead>
<tr>
<th>Gender</th>
<th>Hct (L/L)</th>
<th>Macro%*</th>
<th>RetHct (L/L)</th>
<th>Epo (mIU/mL)</th>
<th>sTfR (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F N=425, 424*</td>
<td>0.40±0.028</td>
<td>1.27±1.36</td>
<td>6.91x10^3±3.09x10^-3</td>
<td>9.506±4.78</td>
<td>20.15±5.55</td>
</tr>
<tr>
<td>M N=369</td>
<td>0.44±0.026</td>
<td>0.72±0.86</td>
<td>7.56x10^3±2.56x10^-3</td>
<td>9.27±3.72</td>
<td>20.55±5.03</td>
</tr>
</tbody>
</table>

Symbol * indicated that there was one data missing of Macro%

2. Histogram and Normal distribution of the On-model related parameters were provided.
(1). Female Hct

Skewness=0.236 Std.Error=0.118,
Kurtosis=0.383 Std.Error=0.118,
Kolmogorov-Smirnov Test Results:
D_{max}=\max(D^*,D')=\max(0.079,-0.058),
D_{0.01}=1.031/Sqr(425)=0.050,
D_{max}>D_{0.01}, P<0.01,
The hypothesis that Hct values fit a
Gaussian distribution is thus rejected.

(2). Male Hct

Skewness=0.029 Std.Error=0.127,
Kurtosis=0.031 Std.Error=0.127,
Kolmogorov-Smirnov Test Results:
D_{max}=\max(D^*,D')=\max(0.062,-0.083),
D_{0.01}=1.031/Sqr(369)=0.054,
D_{max}>D_{0.01}, P<0.01,
The hypothesis that Hct values fit a
Gaussian distribution is thus rejected.

(3). Femal Macro%

Skewness=2.533 Std.Error=0.119,
Kurtosis=8.934 Std.Error=0.119,
Kolmogorov-Smirnov Test Results:
D_{max}=\max(D^*,D')=\max(0.153,-0.183),
D_{0.01}=1.031/Sqr(424)=0.050,
D_{max}>D_{0.01}, P<0.01,
The hypothesis that Macro% values fit a
Gaussian distribution is thus rejected.
(4). Male Macro%

Skewness=3.244, Std.Error=0.127,
Kurtosis=13.161, Std.Error=0.253,
Kolmogorov-Smirnov Test Results:
$D_{max} = \max(D^+, D^-) = \max(0.223, -0.224)$,
$D_{0.01} = 1.031/Sqr(369) = 0.054$,
$D_{max} > D_{0.01}, P < 0.01$,
The hypothesis that Macro% values fit a
Gaussian distribution is thus rejected.

(5). Female RetHct

Skewness=4.431, Std.Error=0.118,
Kurtosis=0.236, Std.Error=0.118,
Kolmogorov-Smirnov Test Results:
$D_{max} = \max(D^+, D^-) = \max(0.102, -0.087)$,
$D_{0.01} = 1.031/Sqr(429) = 0.050$,
$D_{max} > D_{0.01}, P < 0.01$,
The hypothesis that RetHct values fit a
Gaussian distribution is thus rejected.

(6). Male RetHct

Skewness=0.319, Std.Error=0.127,
Kurtosis=0.658, Std.Error=0.253,
Kolmogorov-Smirnov Test Results:
$D_{max} = \max(D^+, D^-) = \max(0.058, -0.032)$,
$D_{0.01} = 1.031/Sqr(369) = 0.054$,
$D_{max} > D_{0.01}, P < 0.01$,
The hypothesis that RetHct values fit a
Gaussian distribution is thus rejected.
(7). Female Epo

Skewness=2.673, Std.Error=0.118,
Kurtosis=0.236, Std.Error=0.118,
Kolmogorov-Smirnov Test results:
\[ D_{\text{max}} = \max(D^+, D^-) = \max(0.125, -0.089), \]
\[ D_{0.01} = 1.031 / \sqrt{425} = 0.050, \]
\[ D_{\text{max}} > D_{0.01}, P < 0.01, \]
The hypothesis that Epo values fit a Gaussian distribution is thus rejected.

(8). Male Epo

Skewness=1.770, Std.Error=0.127,
Kurtosis=7.500, Std.Error=0.253,
Kolmogorov-Smirnov Test results:
\[ D_{\text{max}} = \max(D^+, D^-) = \max(0.113, -0.059), \]
\[ D_{0.01} = 1.031 / \sqrt{369} = 0.054, \]
\[ D_{\text{max}} > D_{0.01}, P < 0.01, \]
The hypothesis that Epo values fit a Gaussian distribution is thus rejected.

(9). Female sTfR

Skewness=1.011, Std.Error=0.118,
Kurtosis=0.236, Std.Error=0.118,
Kolmogorov-Smirnov Test results:
\[ D_{\text{max}} = \max(D^+, D^-) = \max(0.065, -0.041), \]
\[ D_{0.01} = 1.031 / \sqrt{425} = 0.050, \]
\[ D_{\text{max}} > D_{0.01}, P < 0.01, \]
The hypothesis that Sfrr values fit a Gaussian distribution is thus rejected.
(10). Male sTfR

Since all the parameters' distribution did not fit a Gaussian distribution, we used natural logarithms to transform the parameters.[3] However, even after transformed, the variable's distribution did not fit Gaussian distribution. The following was an example.

Example: Ln(EPO)

After transformed:

(I) Kolmogorov-SmirnovTest esults:
\[ D_{\max} = \max(D^+, D^-) = \max(0.049, -0.044) \]
\[ D_{0.01} = 1.031 / \text{Sqr}(369) = 0.054 \]
\[ D_{0.05} = 0.886 / \text{Sqr}(369) = 0.046 \]
\[ 0.05 < D_{\max} < D_{0.01} \]

The hypothesis that Stfr values fit a Gaussian distribution is thus rejected at level 0.05.

(II) Skewness = -0.230, Std.Error = 0.127, Kurtosis = 1.206, Std.Error = 0.253, 
Sk > 2.6, also rejected the Gaussian distribution.
Therefore, we used non-parametric methods to develop the 95% reference ranges, results see Table 4 and 5.

Table 4. Reference ranges and confidence intervals.

<table>
<thead>
<tr>
<th>Female Parameter</th>
<th>2.5% Percentile</th>
<th>90% confid. Lower</th>
<th>90% confid. Upper</th>
<th>97.5% Percentile</th>
<th>90% Confid. Lower</th>
<th>90% Confid. Upper</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct(L/L)</td>
<td>0.35</td>
<td>0.34</td>
<td>0.35</td>
<td>0.45</td>
<td>0.45</td>
<td>0.46</td>
<td>425</td>
</tr>
<tr>
<td>Macro%</td>
<td>0.10</td>
<td>0.00</td>
<td>0.10</td>
<td>5.10</td>
<td>4.10</td>
<td>6.20</td>
<td>424</td>
</tr>
<tr>
<td>RetHct (L/L)</td>
<td>3.13x10^3</td>
<td>2.45x10^3</td>
<td>3.24x10^3</td>
<td>1.32x10^2</td>
<td>1.21x10^2</td>
<td>1.34x10^2</td>
<td>425</td>
</tr>
<tr>
<td>EPO (mIU/mL)</td>
<td>3.40</td>
<td>2.80</td>
<td>4.05</td>
<td>20.70</td>
<td>18.65</td>
<td>22.65</td>
<td>425</td>
</tr>
<tr>
<td>sTfR (nmol/L)</td>
<td>11.22</td>
<td>10.54</td>
<td>12.16</td>
<td>33.38</td>
<td>32.16</td>
<td>34.73</td>
<td>425</td>
</tr>
</tbody>
</table>

Table 5. Reference ranges and confidence intervals.

<table>
<thead>
<tr>
<th>Male Parameter</th>
<th>2.5% Percentile</th>
<th>90% confid. Lower</th>
<th>90% confid. Upper</th>
<th>97.5% Percentile</th>
<th>90% Confid. Lower</th>
<th>90% Confid. Upper</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct(L/L)</td>
<td>0.39</td>
<td>0.39</td>
<td>0.40</td>
<td>0.50</td>
<td>0.49</td>
<td>0.50</td>
<td>369</td>
</tr>
<tr>
<td>Macro%</td>
<td>0.10</td>
<td>0.00</td>
<td>0.10</td>
<td>3.60</td>
<td>2.70</td>
<td>4.40</td>
<td>369</td>
</tr>
<tr>
<td>RetHct (L/L)</td>
<td>2.86x10^-3</td>
<td>1.27x10^-3</td>
<td>3.74x10^-3</td>
<td>1.29x10^-2</td>
<td>1.23x10^-2</td>
<td>1.39x10^-2</td>
<td>369</td>
</tr>
<tr>
<td>EPO (mIU/mL)</td>
<td>3.70</td>
<td>2.75</td>
<td>4.40</td>
<td>18.40</td>
<td>17.20</td>
<td>19.80</td>
<td>369</td>
</tr>
<tr>
<td>sTfR (nmol/L)</td>
<td>12.57</td>
<td>12.16</td>
<td>13.24</td>
<td>32.70</td>
<td>31.22</td>
<td>35.00</td>
<td>369</td>
</tr>
</tbody>
</table>

Discussion

(1) The possible reasons of non-Gaussian distribution of haematological parameters

The exact reason for such a non-Gaussian distribution is not clear, yet it is also noted that the T/E ratio of the athletes is non-Gaussian distribution. From the histograms, we noticed that the skewness was mainly caused by the highest outliers. These outliers may be influenced by many factors including physiological or pathological conditions such as exercise[4], environmental factors[5] and anemia. The reasons also may be caused by non-randomly sampling.
(2) The Chinese athletes’ reference ranges compared with that of foreign elite athletes

The hematological parameters’ reference ranges of Chinese athletes were consistent with those reported by Australian researchers, with the ranges of female Hct (%) 34.3-45.0, RetHct (%) 0.30-1.08, Macro% 0.0-1.9 (after log-transformed), EPO (mU/mL) 4.4-23.5, sTfr (mg/L) 0.76-2.02 (after square root of the value) respectively; and with the ranges of male Hct (%) 38.8-49.6, RetHct (%) 0.31-1.12, Macro% 0.0-1.1 (after log-transformed), EPO (mU/mL) 4.6-18.6, sTfr (mg/L) 0.81-1.87 (after square root of the value) respectively.[6]

(3) Other comments

When calculating the ON-model, the unit of sTfr must be mg/L, since the results were expressed by nmol/L using the R&D ELISA kit, therefore we transformed the unit to match the Dade Behring sTfr[3]. The data presented in this paper were selected using the cut-offs (ON-model <2.5 male, ON-model <2.4 female). In our routine blood tests, once the result exceeded the cut-offs, urine sample should be analyzed to confirm the rhEPO.