Dynamics and Classification of Anthropometric, Physiological and Biochemical Parameters in Adolescent Athletes

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Abstract

Objectives: To date, no correlation has been found between anthropometric parameters determined by blood and urine tests and cardiac morphology. The aim of the study to examine the specific relationships among young male soccer players, combining hematological and biochemical parameters, urine density and composition, heart rate (HR), respiratory rate (RR), and left ventricular morphology with maturity offset (MO) using Z-scores for height-for-age and BMI-for-age. Methods: The relationship between anthropometric, hematological and biochemical parameters was assessed using Spearmen's correlation coefficient, Principal Component Analysis (PCA) and ROC analysis in 404 young male soccer players aged 8–19 years old (12.5±2.5 years old) living in Saint Petersburg, Russia. Results: When conducting Factor analysis, 10 factors were discovered (eigenvalue >1) explaining 65.67% of the total variance. Factor 1 explained 22.16% of the total variance it included the following indicators: handgrip strength, urea, lactate dehydrogenase (LDH), left ventricle (LV), left ventricular internal dimension (LVIDs), interventricular septum (IVS). Factor 2 explained 8.10% of the total variance it included the following variables: red blood cells (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). Factor 3 explained 6.65% of the total variance, contained R-R interval (RR) and heart rate (HR). Factors 4-10 did not have significant variables with factor loadings. According to the ROC analysis handgrip strength, hemoglobin (Hb), RR, HR, and IVS were associated with late maturation (MO below -4.43). The values of handgrip strength, and IVS were also associated with early maturation (MO -1.70 and above). LDH, LV, and LVIDs were associated with underweight (BMI-for-age Z score < -1). Conclusions: This research

model will allow for the development of a program for diagnosing the health of soccer players and determinate the individual level of possible physical activity for each soccer player using MO and the levels of blood parameters.

Keywords: maturity offset; height-for-age; BMI-for-ages Z scores; young male soccer players; hematological parameters; biochemical parameters; instrumental ECG parameters

1. INTRODUCTION

Soccer is one of the most popular and spectator sports in the world [1]. According to the International Federation of Football Associations (FIFA), there are more than 300,000 soccer clubs and 240 million soccer players worldwide [2]. This sport is characterized by repeated episodes of high-intensity short and long sprints, alternating acceleration and deceleration, changes in direction requiring eccentric muscular effort from the athlete, high endurance to aerobic and anaerobic loads, and the development of general, specific physical skills [3]. Therefore, athletes must constantly monitor their physical health [4], especially in youth sports teams, where competition leads adolescents to neglect health care, increasing the risk of injury and overexertion [5].

Various countries have national protocols and standards for the clinical examination of young athletes. In the Russian Federation, this protocol includes monitoring of hematological and biochemical blood parameters, urinalysis, and an anthropometric profile. Hematological parameters (red, white blood cells, platelet count) indicate potential age- and exercise-related changes in oxygen capacity [6] and the risk of anemia [7] due to decreased iron levels, platelet dysfunction [8]. Blood biochemical parameters include creatinine, and lactate dehydrogenase (LDH), which are markers of muscle microdamage and fatigue [6,9], cholesterol, glucose, and total protein to determine nutritional status, malnutrition, or obesity, as well as cardiometabolic and diabetic risks [6,10], urea as a marker of overtraining [6].

Heart rate and RR interval measurements on the electrocardiogram (ECG) reveal the risk of decreased parasympathetic tone, typical for athletes and increased sympathetic activity to control overtraining [11, 12].

Cardiac muscle maturation is characterized by structural, genetic, metabolic and functional changes associated with the transformation of fetal into mature cardiomyocytes [13]. Immature cardiomyocytes are proliferated, and their sarcomeres are not clearly ordered, which is associated with increased expression of fetal cardiomyocyte genes. During the maturation ventricular, atrial and nodal cardiomyocytes undergo various changes. However, during the development of cardiomyocytes from pluripotent stem cells, occur defects of the maturations to development of cardiac diseases due to disruption of the structure (sarcomeres, cytoskeleton, mitochondria) and myocardial function (decreased of proliferation, expression of ion channel proteins). Sarcomere maturation is associated with the alignment of sarcomeric filaments and myomesin. This leads to an increase in the width of the Z- and M-lines of the sarcomere. During myofibril maturation, a switch in sarcomeric protein isoforms from fetal to mature is observed due to post-transcriptional changes and alternative splicing (MYH6 and MYH7 isoforms of myosin are synthesized in fetal and mature cardiomyocytes respec-tively). [13]. Electrophysiological defects in the maturation of cardiomyocytes derived from pluripotent stem cells lead to an arrhythmogenic risk during cell replacement therapy [13, 14].

Anthropometric profiles of football players not only provide information on the dynamics of a child's development but also help to assess the individual characteristics of a player's physical development [15]. Physical development parameters such as height, body weight, body mass index, leg length, and somatotype are associated with power, striking speed, performance, endurance, and body weight control [16]. Anthropometric profiles are usually supplemented by measurements of grip strength, which is associated with muscle strength development [17].

Measuring sitting height helps to assess maturity, which is based on predicting the age of peak height velocity (PHV), the period of time during which an adolescent experience the most rapid upward growth, and the time before or after the PHV of the current chronological age [18]. In 2006, the World Health Organization (WHO) released universal child growth standards to describe optimal growth in children [19]. Z-scores for height-for-age and BMI-for-age were calculated using a reference group of children from almost all over the world living in favorable conditions [19]. WHO Z-score standards are widely recognized as the best system for analyzing and reporting anthropo-metric data and are the most appropriate descriptor of undernutrition and overweight [20]. Identifying predictors among blood parameters and anthropometric data with maturity offset (MO) will allow for individualizing athletes' training loads and training sessions based on their level of physical health, which will improve their performance in games. When MO coincides with athletes' level of physical health, they demonstrate better explosive strength, speed, and agility performance, better cognitive abilities, greater self-confidence, reduced anxiety, less conflict, a lower likelihood of injury during a match [19, 21].

Maturity offset and peak height velocity (PHV) were estimated using the anthropometric parameters proposed by Mirwald et al. [22]. This model is the best way to predict PHV and maturity offset in soccer players and other athletes over 20 years. Most current models include the relationship between only one type of parameter and maturity level, such as muscle damage markers, LV morphology, VO2max [5], and grip strength [23, 24, 25]. To date, no model of biological maturation of MO takes into account all of these parameters, the assessment of which will allow us to study the dynamics of athletes' physical fitness and adjust their workloads, which would improve their performance in games.

Therefore, the aim of the study to examine the specific relationships among young male soccer players, combining hematological and biochemical parameters, urine density and composition, heart rate (HR), respiratory rate (RR), and left ventricular morphology with maturity offset (MO) using Z-scores for height-for-age and BMI-for-age.

2. MATERIAL AND METHODS

2.1.Subjects and study protocol

2.1.1. Subjects

The study included 404 young male athletes (aged 12.5±2.5 years old, 8-19 years), according to the following inclusion criteria: residence since birth in St. Petersburg, practicing only one sport, training at least 2 times a week for 1-1.5 hours for younger and 3-4 times a week for 1.5-2 hours for older athletes, no somatic diseases, bad habits, increased blood pressure and heart rate at rest or inadequate hemodynamic response to physical activity, no injuries and frequent respiratory viral diseases and allergic reactions, and voluntary consent to participate in the study. The exclusion criteria were as follows: newcomers, aged 8 and >19 years, training with breaks or less than 2 training hours per week, practicing more than one sport at the same time, even of the same focus, somatic diseases, frequent colds, allergic diseases, and refusal to sign voluntary informed consent.

2.1.2. Study protocol

We conducted a retrospective analysis of 523 medical control cards of athletes containing the results of a recent medical examination: sociodemographic characteristics, information on lifestyle and health, training load, results of laboratory, instrumental, and anthropometric studies (Fig. 1) according to the list set out in the order of the Ministry of Health of the Russian Federation No. 1144n of October 23, 2020. Accordingly, 438 soccer players who met the main inclusion criteria were selected. Then, another 34 records were excluded according to additional criteria: lack of the entire list of examinations and medical examination the day after training (on average 20 hours after the last loading) to exclude the influence of fatigue.

This study was conducted according to the Good Clinical Practice Guidelines of the Declaration of Helsinki. This study was approved by the local ethical committee of St. Petersburg State Pediatric Medical University (protocol no. 65/06, February 20, 2026). All participants and their parents/guardians provided informed consent.

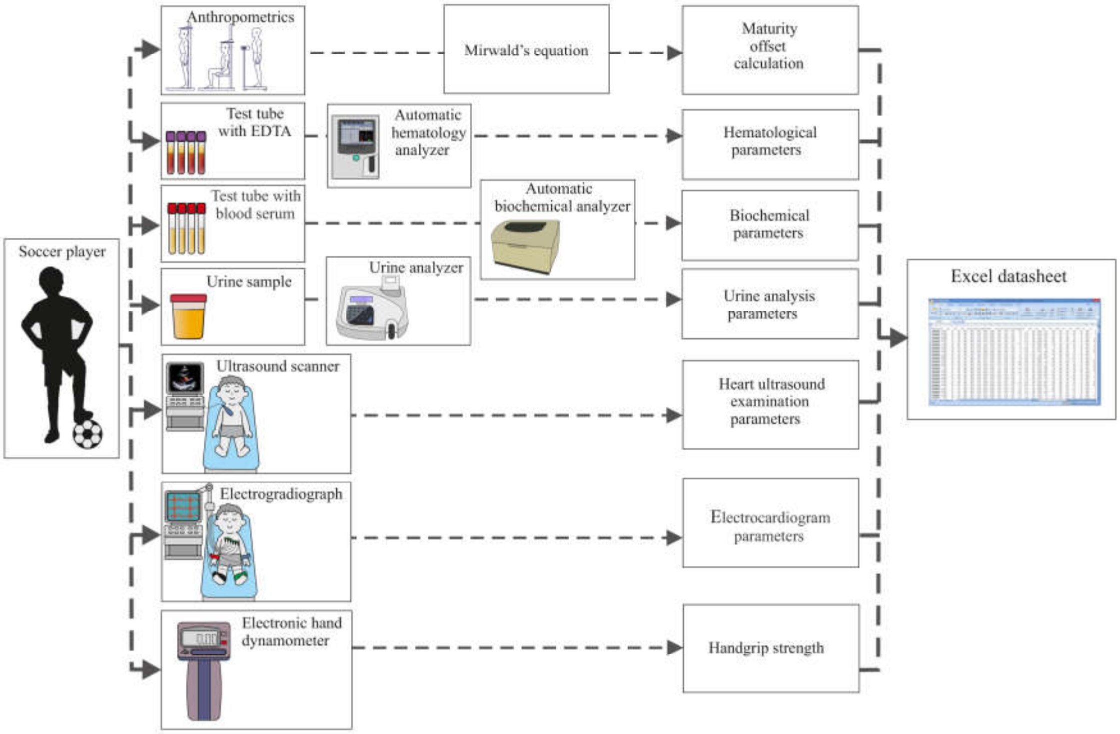


Figure 1. Graphic presentation of the study protocol.

2.2.Anthropometry and assessment of maturity offset

Anthropometric measurements were performed in a separate medical room with artificial lighting under comfortable environmental conditions (temperature, 18°C–20°C; humidity, 50%–60%). Participants were barefoot and wore light underwear without sleeves. Stature and sitting height were measured using a metal stadiometer RM-2-«Diakoms» (Moscow, Russia) with a movable spring-loaded clamp, two rulers, and a folding seat with a measurement accuracy of 0.5 cm. For stature assessment, the subject was positioned in the standard erect posture with the head in Frankfort's horizontal plane. The subject was asked to sit erect on a bench 50 cm high with the head in the Frankfort horizontal plane to measure the sitting height. The difference between the stature and sitting height provided the leg length. Body weight was assessed using electronic medical scales VEM-150.3-A3, «MASSA-K» (Saint Petersburg, Russia) with a measurement accuracy of 50 g. The measurement error of the metal stadiometer RM-2-«Diakoms» (Moscow, Russia) is no more than 0.5 cm; the error of the medical scales VEM-150.3-A3, «MASSA-K» (Saint Petersburg, Russia) is up to 75 g.

Height-for-age and BMI-for-age Z-scores were analyzed using the World Health Organization (WHO) AntroPlus 1.0.4 (World Health Organization, Switzerland, Geneva, 2009) software for the global application of the WHO Reference for 5-19 years to monitor the growth of schoolage children and adolescents. MO was calculated using Mirwald’s equations (formula 1) based on the obtained anthropometric parameters, where SiH is the sitting height, LL is leg length; BW is body weight, and StH is stature [22]. As stated earlier, this equation is robust to individual differences in maturation rate, unlike newer equations such as Moore's model.

$$\text{Maturity offset} = -9{,}236 + 0{,}0002708 \times \frac{\text{SiH}}{\text{LL}} - 0{,}001663 \times \frac{\text{Age}}{\text{LL}} + 0{,}007216 \times \frac{\text{Age}}{\text{SiH}} + 0{,}02292 \times \frac{\text{BW}}{\text{StH}} \quad (1)$$

2.3.Laboratory tests

Venous blood samples were collected between 7:00 and 8:30 a.m. following an overnight fast. Samples of 7–10 ml were drawn into a vacuum tube. Blood samples were separated by placing 2.5 ml of blood into a tube with EDTA for hematology analysis and 4.5-7.5 ml of whole blood. Whole blood was centrifuged at 1500 rpm for 10 min within 20 min of collection to separate serum from other components for subsequent measurements. Hematological parameters – red blood cells (RBC), white blood cells (WBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets count (PLT) was recorded using the URIT-5160 automatic hematology analyzer (URIT Medical Electronics Co. Ltd, Guilin, China). Plasma biochemical parameters – alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase (AML), creatine kinase (CK), lactate dehydrogenase (LDH), cholesterol (Chol), glucose (Glu), total protein (TProt), total bilirubin (TBIL), creatinine (CRE, blood), urea, iron was measured using a Mindray BS-200E biochemical analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). Each participant collected a 30-mL morning urine sample of 30-50 ml in a special container. The specific gravity of urine (SG), urine, creatinine (CRE [urea]), pH, and microalbumin (MALB) concentration were measured using a DIRUI H-100 urine analyzer (DIRUI lndustrial, Ltd., Changchun, China).

The hematological clinical and biochemical studies included in-house quality control. All laboratory procedures were conducted in strict compliance with the conditions and recommendations of the manufacturers regarding storage conditions and expiration dates of test systems and chemical reagents. Hematological studies were conducted only on meteorologically verified, certified equipment and software. Blood samples with hemolysis were excluded from the studies. The variability of cell, protein, and blood parameters was assessed in accordance with the reference range of values pro-vided by the test system manufacturers and the range of values of the hematological analyzers. Values of parameters in samples that were outside the reference range of the test system were excluded from the study.

Measurements were performed in triplicate, after which the mean value for each analysis per patient was calculated. The results were evaluated using the coefficient of variation (CV, %).

2.4.ECG, cardiac ultrasound, dynamometry

A continuous 12-channel recorder «Computer Electrocardiograph Valenta EKGK-02» (Neo Company, Ltd, Russia) with a sampling frequency of 2000 Hz was used to record the electrocardiogram (ECG). Each subject had an ECG recorded in standard, enhanced, and chest leads for at least 3 minutes from 10:00 to 13:00 in a separate equipped medical office in a lying position after 1-3 minutes of adaptation. The average heart rate (HR) and RR-interval (RR) were calculated using the «Valenta 1.4» software installed on a personal computer.

Ultrasound heart' investigation with determination of the dimensions of the left ventricle (LV), left ventricular internal dimension in systole (LVIDs), ejection fraction (EF), thickness of the interventricular septum (IVS) was performed in a special medical office using the ultrasound scanner «RuScan 65» (Scientific and Production Association «SCANNER», Russia) and a sector sensor with a frequency of 1.5-5 MHz in the supine position after 1-3 minutes of adaptation and normalization of breathing. The ultrasound examinations were performed by one certified specialist with more than 20 years of experience.

The handgrip strength of each hand was measured by an electronic hand dynamometer DMER-30-0.5 (JSC Tulinovsky Instrument-Making Plant TVES, Russia) with an accuracy of 0.5 kg. The subject was instructed to stand straight, the arm was lowered along the body, not touching it, the elbow was slightly bent. The test was repeated for each hand three times with a pause of one minute between each test to avoid the effect of muscle fatigue. The best (maximum) result for each hand was saved. The average of the maxima for both handgrip strength was used for the analysis.

2.5.Statistical Analysis

In the first stage of the analysis, The Shapiro-Wilk test was used to tested whether the data came from a normally distributed sample. It showed $P<0.05$, which explains the correlation analysis was perform using the Spearman's rank correlation coefficient (ρ). To adjust the significance level (α) for multiple statistical comparisons, the Bonferroni correction was used, which takes into account the number of tests. Each individual test met a more stringent criterion: $p < \alpha/m$, where α is the baseline level (e.g., 0.05) and m is the number of tests.

To reduce the number of potential hematological and biochemical parameters associated with height-for-age and BMI-for-age Z-scores, factor analysis was performed using principal component analysis (PCA) for factor extraction using Statistica data analysis software version 10, StatSoft Inc., USA, 2011 [26]. As a result, eigenvalues and factor loadings of each component were calculated. Factors, explaining the maximum percentage of total variance were selected based on the Kaiser criterion (eigenvalue > 1). Variables with factor loadings > 0.7 were considered to have the largest contribution.

All volunteers were divided into 2 groups: late maturing (MO < -4.43 years (the value corresponds to the threshold of <25th percentile). normally maturing (MO from -4.43 to -1.70 years). and early maturing (MO > -1.70 years (the value corresponds to the threshold of >75th percentile). Body weight and stature deviations were diagnosed using the generally accepted WHO Child Growth Standards. The stunted and under-weight were diagnosed at height-for-age and BMI-for-age Z scores < -1. tallness and overweight – at Height-for-age and BMI-for-age Z scores > 1. normal height and weight – at height-for-age and BMI-for-age Z scores from -1 to 1.

ROC analysis was performed to determine the prognostic effectiveness of hematological. biochemical. and instrumental parameters in relation to maturation offset and physical development. The area under the curve (AUC). predictor threshold values. sensitivity and specificity of predictors. and likelihood ratio (LR) were performed blood and instrumental parameters were used to determine predictive efficiency. and their sensitivity (Se) and specificity (Sp) were calculated as shown in Formula 2:

$$Se = \frac{a}{(a + c)} \times 100\% \quad (2)$$

where Se is the sensitivity. a is the true positive result – the number of parameter values were above the laboratory and instrumental predictor threshold value in a group of soccer player with early and late maturing. as soon as risk of stunted and underweight. tallness. and overweight; and c is the false negative result is the number of the value of parameters above the threshold in a group of soccer player with early and late maturing. as soon as risk of stunted and underweight, tallness, and overweight.

Sensitivity shows the number of true positive results in the group of athletes. Specificity (Sp) were calculated using Formula 3. Specificity shows the number of true negative results in the group of athletes.

$$Sp = \frac{d}{(b + d)} \times 100\% \quad (3)$$

where b is the false-positive result. which is the number of parameter values being below the threshold in a group of soccer players in a group of soccer player with early and late maturing. as soon as risk of stunted and underweight, tallness, and overweight; is the true-negative result, which is the number of parameter values were below the threshold in a group of soccer player with early and late maturing as soon as risk of stunted and underweight.

To interpret the AUC values, we used the following criteria: 0.7-0.8: is considered acceptable; 0.8-0.9: is considered excellent; greater than 0.9: is considered outstanding according to the source [27]. Thus, in the article we left the AUC data only for those parameters whose AUC was higher than 0.69 and we deleted the remaining parameters.

Descriptive statistics. correlation analysis and ROC analysis were performed using GraphPad Prism version 8.01. 09.21.2020. San Diego. CA, USA. Data are presented as the mean and 95% CI and were considered significant at $p < 0.05$.

3. RESULTS

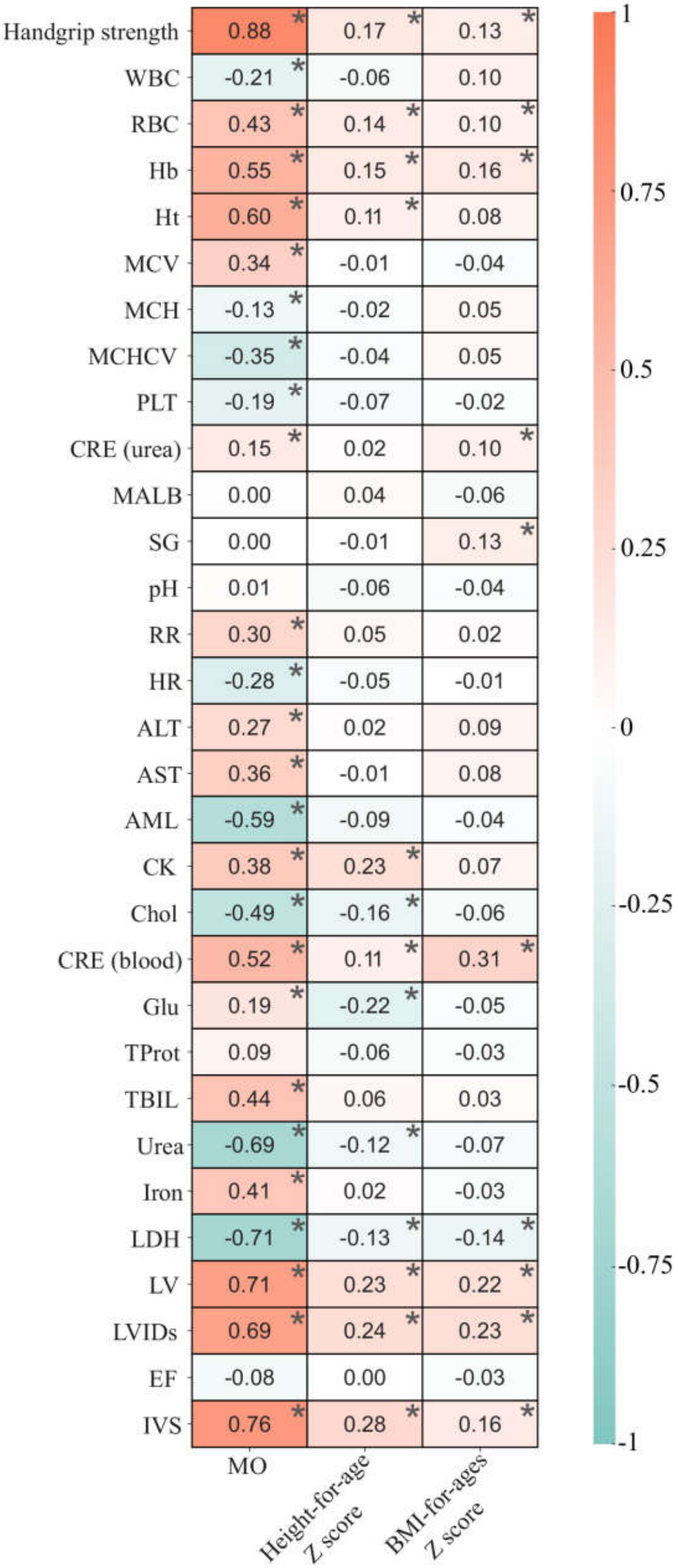
The descriptive statistics of hematological. biochemical. and instrumental parameters are presented in Appendix (Table 2). The MO had strong direct correlation with LVIDs. IVS. handgrip strength and CRE (blood) (Fig. 2). The BMI-for-age Z score had moderate direct correlation with CRE (blood) and LDH (Fig. 2).

Factor analysis selected 10 components (eigenvalue > 1) explaining 65.67% of the total variance (Tab. 1, fig. 3). Factor 1 explained 22.16% of the total variance, the highest factor loading was possessed by: handgrip strength (0.79), urea (-0.72), LDH (-0.75), LV (0.78), LVIDs (0.76), IVS (0.81), Fig. 4. Factor 2 explained 8.10% of the total variance and had the following variables with the maximum factor loading: RBC (-0.74), MCH (0.74), MCHC (0.77), Fig. 4. Factor 3 explained 6.65% of the total variance, contained RR (0.80) and HR (0.81) with the maximum factor loading (Fig. 4). Factors 4-10 did not have significant variables with factor loadings.

Table 1. Eigenvalue for 10 factors

Factor	Eigenvalue	% of total variance
Factor 1	6.869654	22.16018
Factor 2	2.511434	8.10140
Factor 3	2.060021	6.64523
Factor 4	1.561326	5.03654
Factor 5	1.334408	4.30454
Factor 6	1.311568	4.23086
Factor 7	1.303882	4.20607
Factor 8	1.190275	3.83960
Factor 9	1.141302	3.68162
Factor 10	1.075366	3.46892

Total		65.6695



	MO	Height-for-age Z score	BMI-for-ages Z score
Handgrip strength	0.88 *	0.17 *	0.13 *
WBC	-0.21 *	-0.06	0.10
RBC	0.43 *	0.14 *	0.10 *
Hb	0.55 *	0.15 *	0.16 *
Ht	0.60 *	0.11 *	0.08
MCV	0.34 *	-0.01	-0.04
MCH	-0.13 *	-0.02	0.05
MCHCV	-0.35 *	-0.04	0.05
PLT	-0.19 *	-0.07	-0.02
CRE (urea)	0.15 *	0.02	0.10 *
MALB	0.00	0.04	-0.06
SG	0.00	-0.01	0.13 *
pH	0.01	-0.06	-0.04
RR	0.30 *	0.05	0.02
HR	-0.28 *	-0.05	-0.01
ALT	0.27 *	0.02	0.09
AST	0.36 *	-0.01	0.08
AML	-0.59 *	-0.09	-0.04
CK	0.38 *	0.23 *	0.07
Chol	-0.49 *	-0.16 *	-0.06
CRE (blood)	0.52 *	0.11 *	0.31 *
Glu	0.19 *	-0.22 *	-0.05
TProt	0.09	-0.06	-0.03
TBIL	0.44 *	0.06	0.03
Urea	-0.69 *	-0.12 *	-0.07
Iron	0.41 *	0.02	-0.03
LDH	-0.71 *	-0.13 *	-0.14 *
LV	0.71 *	0.23 *	0.22 *
LVIDs	0.69 *	0.24 *	0.23 *
EF	-0.08	0.00	-0.03
IVS	0.76 *	0.28 *	0.16 *

Figure 2. Correlation heat map for the factors considered for the prediction of MO, height-for-age and BMI-for-ages Z scores. The heatmap displays correlation coefficients. The color intensity on the heatmap scale reflects the strength of the correlations. Note. * p<0.0001.

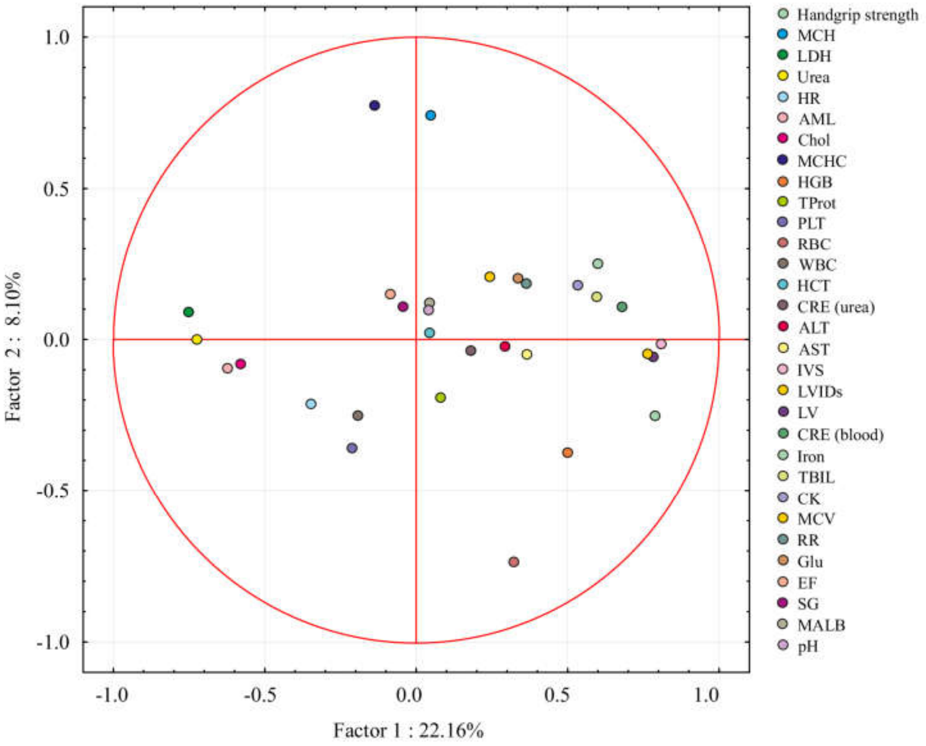


Figure 3. Principal component analysis biplot for all variables.

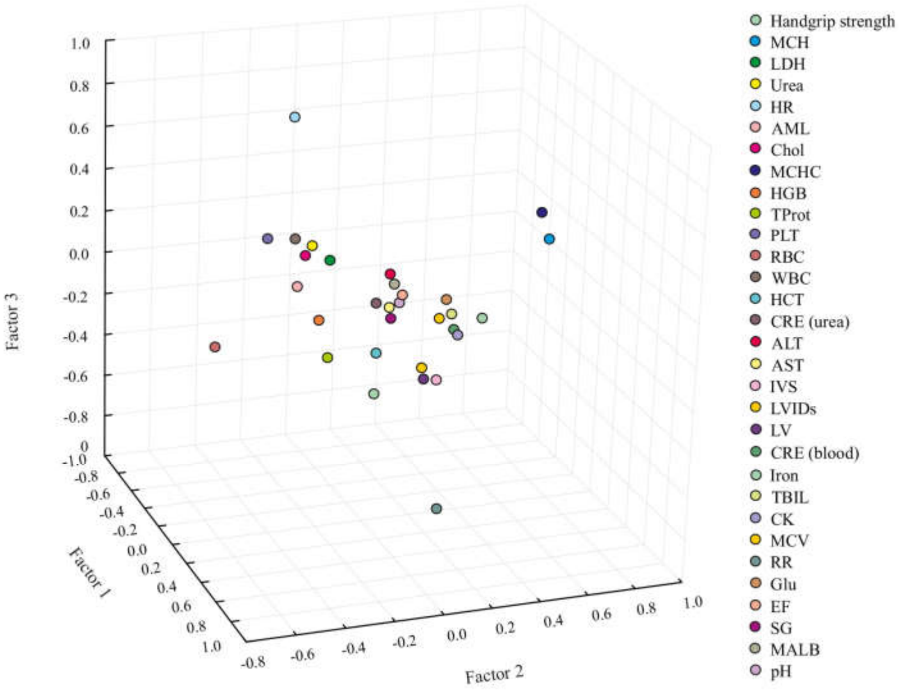


Figure 4. 3D plot of factor loadings for the first three factors.

According to the ROC analysis results presented in Appendix (Table 3) the values of handgrip strength < 16.75 kg, Hb < 132.50 g/L, RR < 0.84 ms, HR > 71.50, and IVS < 6.90 mm associated with late maturation (MO below -4.43).

The values of handgrip strength > 26.75 kg, and IVS > 7.75 mm associated with early maturation (MO -1.70 and above (Table 3).

As shown in Table 5 LDH > 389.5 U/L, LV<46.50 mm, and LVIDs < 46.50 mm were associated with underweight (BMI-for-age Z score < -1). None of the laboratory and instrumental parameters were associated with overweight (BMI-for-age Z score > 1).

To test the stability of the model internal validation was performed using k-means cluster analysis, Table 6. Since all patients were initially divided into 3 clusters using cluster analysis according to the MO threshold values. The first cluster included patients with MO greater than -1.70, the second from -1.72 to -4.73, and the third group from -4.73 to -5.73. Validation was performed within each cluster for the AUC parameters whose values were greater than 0.7. These parameters included: handgrip strength. Hb, RR, HR, IVS, as well as LDH, LV, LIVDs Tables 3, 5. Then. the accuracy (sensitivity and specificity) of each parameter was assessed in groups 1 and 2 and compared with group 3. which served as a control. After that, the accuracy was assessed in groups 1 and 3, and group 2 served as a control. Accuracy was also assessed in groups 2 and 3, with group 1 used as a control. The lower validation accuracy (AUC) of the k-means model is explained by the smaller number of athletes included in each model cluster.

4. DISCUSSION

The main findings of this study were to elucidate laboratory and clinical parameters associated with early and late maturation, as well as growth (height-for-age Z > 1) and underweight (BMI-for-age Z < -1). Factor analysis revealed that Factor 1, which explained 22.16% of the total variance, was primarily associated with a combination of instrumental parameters characterizing grip strength, cardiac morphometric features, and biochemical status. Factor 2 explained 8.10% of the total variance and was associated with hematological parameters. Factor 3, which explained 6.64% of the total variance, characterized HR and RR, and was possibly associated with vegetative status.

Our results confirm previous studies showing a positive correlation between grip strength and MO [28]. Ploegmakers et al., Ramos et al. and Zarate-Osuna et al. showed body weight and height were highly correlated with MO in European and Brazilian children and adolescents aged 3 to 16 years [29,30,31]. In our study. grip strength was associated with late and early maturation, Table 2,3. The inclusion of grip strength in the maturation rate and anthropometric profile model was unexpected. as soccer players do not use their hands in training or in practicing motor skills associated with them [17,32]. Thus. grip strength may be related to the general and specific physical fitness of soccer players.

Among the hematological parameters associated with early and late MO in soccer players. Hb was noted, Table 3. Blood oxygen-carrying capacity determined by Hb concentration may be a predictor of performance. This leads to physiological hyper-chromia and an increase in VO2 max in boys during and shortly before puberty [33]. The physiological mechanisms underlying the increase of Hb concentration are the in-creasing influence of growth hormone and testosterone on red blood cell hematopoiesis [34,35]. Several studies have shown that Hb concentration is associated with accelerated linear growth, VO2max, and oxygen capacity [5,25,36].

In the present study, an association between MO and IVS was found (Fig. 4). Also, in the study by Perkins D.R et al. a predictive model of a positive association between left ventricular size, Hb concentration, and VO2max with MO in trained boys was developed [24]. The IVS turned out to be a significant factor in somatic maturity in soccer players with early MO. Taylor et al. and Chirinos et al. found an association between body length and left ventricular volume [37,38]. Significant growth of the heart occurs during maturation, physical training during puberty leads to significant cardiac remodeling and an increase its size [39].

This study demonstrated for the first time a reliable relationship between the level of RR in early-maturing and late-maturing soccer players (Table 3). Measurement of RR are associated with VO2max and are markers of aerobic metabolism and fatigue in athletes [40]. During moderate-intensity exercise (50-60% of VO2 max), the influence of the vagus nerve ceases and sympathetic tone begins to predominate [41]. Sympathetic innervation at rest and during heart contractions predominates in young children, which is one of the factors limiting their aerobic performance during training [25]. Sympathovagal balance gradually develops with age towards the predominance of parasympathetic control and a decrease in heart rate at rest, which creates conditions for a wider range of cardiac adaptation to stress [25]. Rapid expansion of the cardiac chambers in combination with the dominance of the sympathetic nervous system can lead to arrhythmias and ischemia with possible dramatic consequences [42]. Thus, the relation-ship between MO level and cardiac autonomic regulation can be considered an important factor determining the modulation of vagal tone.

This study builds on the results of several recent studies, which were significantly limited in the number of parameters considered. Narula et al. and Bellfield et al. applied a classification of electrocardiographic and echocardiographic results to assess the risk of developing “athlete's heart” [46,47].

5. LIMITATIONS

In future studies, the results should be confirmed using validation procedure in independent samples. In addition, the identified associations are limited by the prospect of use only among soccer players. In addition, we were unable to obtain high values of AUC (0.55-0.70). Se and Sp (53%-70%) for most parameters except handgrip strength. Another limitation may be the use of the obtained data at low and high (outside the normal range) levels of model indicators. Extrapolation of the obtained results to non-sports children, healthy adults or subjects with diseases is possible only after their validation in relevant groups.

6. CONCLUSIONS

In this study, we first investigated the relationship between MO using Z-scores for age, height-for-age, and BMI-for-age with instrumental ECG and laboratory blood, urine parameters young Russian male soccer players. Low values of grip strength, Hb and IVS, LV, LVID, HR were associated with late maturation in soccer players (MO < -4.43 years). High values of grip strength, Hb, RR, IVS, and values were associated with early maturation (MO > -1.70 years). Underweight (BMI for age Z < -1) was associated with elevated LDH levels, LV, LVIDs. The results of this study indicate the need for joint monitoring of cardiac morphological features and the balance of the sympathetic and para-sympathetic nervous systems in young children. This research model will allow for the development of a program for diagnosing the health of soccer players and determinate the individual level of possible physical activity for each soccer player using MO and the levels of blood parameters.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflicts of interest.

Table 2. Descriptive statistics of hematological, biochemical and instrumental parameters (M; 95% CI).

Parameter	Values
MO, years	-3.10 (-2.26; -2.94)
Height-for-age Z score	0.54 (0.45; 0.63)
BMI-for-age Z score	-0.09 (-0.18; 0.004)
Hematological parameters	
RBC, 10^{12}/L	4.95 (4.89; 5.00)
WBC, 10^{9}/L	6.49 (6.31; 6.67)

Hb, g/L	136.81 (135.69; 137.93)
Ht, %	54.00 (53.80; 54.20)
MCV, fL	85.68 (85.10; 86.26)
MCH, pg	27.15 (26.86; 27.44)
MCHC, g/L	316.78 (3.14.10; 319.46)
PLT, 10^9/L	272.84 (266.72; 278.96)
Serum biochemical parameters	
ALT, U/L	20.78 (2.14; 21.42)
AST, U/L	32.05 (31.19; 32.91)
AML, U/L	171.26 (165.59; 176.93)
CK, U/L	255.91 (241.73; 270.06)
LDH, U/L	384.34 (375.82; 392.85)
Chol, mmol/L	3.67 (3.59; 3.74)
CRE (blood), umol/L	98.42 (96.53; 100.31)
Glu, mmol/L	5.18 (5.13; 100.31)
Total Prot, g/L	74.24 (73.70; 74.78)
TBIL, umol/L	17.41 (16.55; 18.27)
Urea, mmol/L	5.80 (5.61; 5.99)
Iron, umol/L	19.48 (18.88; 20.08)
Urine test parameters	
SG, g/mL	1020.12 (1015.64; 1024.60)
MALB, mg/L	35.02 (30.62; 39.68)
pH	6.28 (6.20; 6.36)
CRE (urea), mmol/L	13.32 (12.51; 14.13)
Instrumental parameters	
Handgrip strength, kg	23.15 (22.22; 24.08))
HR, bpm	70.92 (69.83; 72.01)
RR, sec	0.87 (0.85; 0.88)
LV, mm	47.86 (47.36; 48.35)
LVIDs, mm	29.46 (29.05; 29.86)
EF, %	71.33 (70.65; 72.00)
IVS, mm	7.23 (7.12; 7.33)

Table 3. ROC analyses for threshold values. AUC. sensitivity and specificity its 95%CI of hematological. biochemical and instrumental parameters for early and late maturation prediction.

Parameter	MO category. years	Threshold	AUC	Sensitivity, %	Specificity, %	LR	*p*
Handgrip strength	**< -4.43**	**< 16.75**	**0.90 (0.87;0.93)**	**80.39 (71.65;86.93)**	**81.09 (75.12;85.91)**	**4.25**	**<0.0001**
Handgrip strength	**> -1.70**	**> 26.75**	**0.91 (0.88;0.95)**	**89.11 (81.54;93.81)**	**81.09 (75.12;85.91)**	**4.71**	**<0.0001**
Urea	< -4.43	> 5.85	0.53 (0.46;0.60)	50.0 (40.47;59.53)	54.23 (47.33;60.97)	1.09	0.3973
Urea	> -1.70	> 5.45	0.51 (0.44;0.58)	49.5 (39.95;59.09)	45.77 (39.03;52.67)	0.91	0.9129
LDH	< -4.43	> 386.5	0.56 (0.49;0.63)	55.45 (45.73;64.76)	52.74 (45.85;59.52)	1.087	0.3751
LDH	> -1.70	< 356.5	0.53 (0.46;0.60)	57.84 (48.15;66.97)	46.77 (39.99;53.66)	1.167	0.4122
RBC	< -4.43	> -1.70	0.53 (0.46;0.60)	57.84 (48.15;66.97)	46.77 (39.99;53.66)	1.087	0.2105
RBC	> -1.70	< -4.43	0.53 (0.46;0.60)	50.5 (40.91;60.05)	56.72 (49.80;63.38)	1.167	0.3751
MCH	< -4.43	> -1.70	0.53 (0.46;0.60)	48.04 (38.59;57.63)	57.21 (50.30;63.86)	1.123	0.4122
MCH	> -1.70	< -4.43	0.51 (0.44;0.58)	50.5 (40.91;60.05)	48.26 (41.45;55.13)	0.9759	0.8751
RR	**< -4.43**	**< 0.835**	**0.70 (0.63;0.76)**	**65.69 (56.05;74.18)**	**63.68 (56.83;70.02)**	**1.81**	**<0.0001**
RR	> -1.70	> 0.895	0.58 (0.51;0.65)	57.43 (47.69;66.62)	56.72 (49.80;63.38)	1.33	0.0261
HR	**< -4.43**	**> 71.50**	**0.69 (0.63;0.76)**	**66.67 (57.06;75.06)**	**62.19 (55.32;68.60)**	**1.76**	**<0.0001**

HR	> -1.70	< 67.50	0.56 (0.49; 0.63)	56.44 (46.71; 65.69)	54.73 (47.82; 61.45)	1.25	0.0964
LV	< -4.43	< -4.43	0.60 (0.53; 0.66)	59.8 (50.10; 68.80)	56.22 (49.31; 62.90)	1.366	0.0052
LV	> -1.70	> -1.70	0.52 (0.45; 0.58)	55.45 (45.73; 64.76)	48.76 (41.93; 55.62)	1.082	0.6389
LVID	< -4.43	< -4.43	0.55 (0.48; 0.62)	55.88 (46.21; 65.13)	51.24 (44.38; 58.07)	1.146	0.1605
LVID	> -1.70	> -1.70	0.51 (0.45; 0.58)	53.47 (43.79; 62.89)	48.76 (41.93; 55.62)	1.043	0.6783
IVS	**< -4.43**	**< 6.90**	**0.83 (0.78; 0.87)**	**73.53 (64.23; 81.12)**	**77.61 (71.36; 82.83)**	**3.28**	**<0.0001**
IVS	**> -1.70**	**> 7.75**	**0.80 (0.75; 0.85)**	**85.15 (76.93; 90.79)**	**62.19 (55.32; 68.60)**	**2.25**	**<0.0001**

Note: AUC greater than 0.69 is shown in bold.

Table 4. ROC analyses for threshold values. AUC. sensitivity and specificity and its 95% CI of hematological. biochemical and instrumental parameters for Height-for-age Z score prediction.

Parameter	Z score category. conv. unit	Threshold	AUC	Sensitivity, %	Specificity, %	LR	*p*
Handgrip strength	< -1	< 19.75	0.57 (0.46; 0.68)	52 (33.50; 69.97)	51.54 (45.49; 57.55)	1.07	0.2582
Handgrip strength	> 1	> 21.75	0.59 (0.5316; 0.65)	53.78 (44.85; 62.48)	55.38 (49.31; 61.30)	1.21	0.0044
Urea	< -1	> 5.950	0.51 (0.40; 0.63)	56 (37.07; 73.33)	53.85 (47.77; 59.81)	1.21	0.827
Urea	> 1	< 5.650	0.57 (0.51; 0.63)	59.66 (50.68; 68.04)	52.69 (46.63; 58.68)	1.26	0.0273
LDH	< -1	<351.5	0.53 (0.41; 0.65)	52.00 (33.50; 69.97)	57.31 (51.23; 63.17)	1.218	0.571

LDH	> 1	<371.0	0.54 (0.48; 0.60)	55.46 (46.50; 64.08)	50.77 (44.73; 56.79)	1.127	0.1825
RBC	< -1	> 4.905	0.55 (0.42; 0.67)	52.00 (33.50; 69.97)	56.15 (50.08; 62.05)	1.186	0.4082
RBC	> 1	> 4.895	0.51 (0.45; 0.57)	47.90 (39.13; 56.80)	55.38 (49.31; 61.30)	1.074	0.6913
MCH	< -1	> 27.45	0.52 (0.41; 0.63)	52.00 (33.50; 69.97)	51.54 (45.49; 57.55)	1.073	0.6667
MCH	> 1	< 27.65	0.50 (0.44; 0.56)	51.26 (42.38; 60.06)	44.62 (38.70; 50.69)	0.9255	0.9251
RR	< -1	< 0.8450	0.56 (0.43; 0.69)	60.00 (40.74; 76.60)	55.77 (49.69; 61.68)	1.357	0.278
RR	> 1	> 0.8750	0.51 (0.44; 0.57)	52.10 (43.20; 60.87)	53.85 (47.77; 59.81)	1.129	0.7055
HR	< -1	> 73.50	0.57 (0.44; 0.70)	60.00 (40.74; 76.60)	65.77 (59.81; 71.27)	1.753	0.2132
HR	> 1	< 68.50	0.50 (0.44; 0.57)	51.26 (42.38; 60.06)	54.23 (48.16; 60.18)	1.12	0.7711
LV	< -1	< 29.50	0.60 (0.48; 0.72)	68 (48.41; 82.79)	46.92 (40.95; 52.99)	1.28	0.1056
LV	> 1	> 29.50	0.61 (0.55; 0.67)	60.5 (51.52; 68.83)	53.08 (47.01; 59.05)	1.29	0.0005
LVID	< -1	> 29.50	0.54 (0.42; 0.66)	52.00 (33.50; 69.97)	50.77 (44.73; 56.79)	1.056	0.4737
LVID	> 1	> 29.50	0.50 (0.44; 0.56)	51.26 (42.38; 60.06)	50.77 (44.73; 56.79)	1.041	0.8328
IVS	< -1	> 7.250	0.61 (0.49; 0.73)	56.00 (37.07; 73.33)	60.77 (54.72; 66.51)	1.427	0.0549
IVS	> 1	> 7.250	0.54 (0.48; 0.61)	45.38 (36.72; 54.33)	60.77 (54.72; 66.51)	1.157	0.1212

Table 5. ROC analyses for threshold values. AUC. sensitivity and specificity and its 95%CI of hematological. biochemical and instrumental parameters for BMI-for-age Z score prediction.

Parameter	**Z score category. conv. unit**	**Threshold**	**AUC**	**Sensitivity, %**	**Specificity, %**	**LR**	***p***
Handgrip strength	< -1	< 20.25	0.64 (0.57; 0.71)	69.64 (56.66; 80.10)	54.49 (48.84; 60.02)	1.53	0.001
Handgrip strength	> 1	< 21.25	0.51 (0.43; 0.59)	57.45 (43.28; 70.49)	50.83 (45.21; 56.43)	1.17	0.7564
Urea	< -1	> 5.950	0.55 (0.47; 0.63)	51.79 (39.01; 64.33)	57.81 (52.16; 63.25)	1.227	0.1999
Urea	> 1	> 5.650	0.51 (0.42; 0.59)	48.94 (35.28; 62.76)	53.16 (47.51; 58.72)	1.045	0.8
LDH	**< -1**	**> 389.5**	**0.70 (0.62; 0.77)**	**75.00 (62.31; 84.48)**	**63.12 (57.54; 68.38)**	**2.03**	**<0.0001**
LDH	> 1	> 363.5	0.55 (0.48; 0.63)	63.83 (49.54; 76.03)	55.81 (50.17; 61.32)	1.45	0.2273
RBC	< -1	< 4.725	0.60 (0.52; 0.68)	58.93 (45.88; 70.83)	62.79 (57.20; 68.06)	1.584	0.0145
RBC	> 1	> 4.825	0.51 (0.42; 0.60)	55.32 (41.25; 68.59)	45.18 (39.66; 50.83)	1.009	0.7928
MCH	< -1	> 27.45	0.53 (0.45; 0.61)	53.57 (40.70; 65.98)	53.82 (48.18; 59.37)	1.128	0.4651
MCH	> 1	> 27.55	0.53 (0.45; 0.61)	53.57 (40.70; 65.98)	53.82 (48.18; 59.37)	1.16	0.4651
RR	< -1	< 0.8550	0.56 (0.48; 0.64)	57.14 (44.14; 69.23)	57.48 (51.83; 62.93)	1.344	0.1197
RR	> 1	< 0.8650	0.58 (0.49; 0.66)	59.57 (45.34; 72.36)	53.16 (47.51; 58.72)	1.272	0.0746
HR	< -1	> 71.50	0.56 (0.48; 0.63)	53.57 (40.70; 65.98)	60.47 (54.84; 65.82)	1.355	0.154
HR	> 1	> 70.50	0.58 (0.49; 0.66)	55.32 (41.25; 68.59)	57.14 (51.50; 62.61)	1.291	0.0735
LV	**< -1**	**<46.50**	**0.69 (0.62; 0.76)**	**64.29 (51.19; 75.54)**	**64.78 (59.23; 69.96)**	**1.825**	**0.000005**

LV	**> 1**	**<46.50**	**0.71 (0.61; 0.80)**	**64.29 75.54)**	**(51.19;63.83 76.03)**	**(49.54;**	**1.777**	**0.000337**
LVIDs	**< -1**	**< 46.50**	**0.69 (0.62; 0.76)**	**64.29 75.54)**	**(51.19;64.78 69.96)**	**(59.23;**	**1.83**	**<0.0001**
LVIDs	> 1	> 55.50	0.50 (0.41; 0.59)	10.64 22.59)	(4.63;92.69 95.12)	(89.18;	1.46	0.9621
IVS	< -1	< 6.900	0.62 (0.53; 0.70)	41.07 54.12)	(29.17;71.1 75.93)	(65.73;	1.421	0.006118
IVS	> 1	> 6.650	0.50 (0.42; 0.59)	78.72 88.01)	(65.10;28.24 33.57)	(23.45;	1.097	0.934148

Note: AUC greater than 0.69 is shown in bold.

Table 6. K-means cluster analysis on AUC for model parameters.

Parameter	AUC, p								
	1 group			2 group			3 group		
MO	0.92	0.04	-0.84	-1.73	-2.61	-3.51	-4.43	-4.77	-5.10
Comparisons	1-2	2-3	1-3	1-2	2-3	1-3	1-2	2-3	1-3
handgrip strength	0.6368 0.2271	0.5945 0.1852	0.6944 0.0738	0.6711 0.0845	0.6841 0.4324	0.6189 0.17538	0.5562 0.4706	0.6386 0.0944	0.5728 0.4453
RR	0.5671 0.5578	0.5215 0.7568	0.5609 0.5819	0.5133 0.7247	0.5380 0.3691	0.5232 0.5733	0.5969 0.2263	0.6710 0.0611	0.6235 0.0744
HR	0.6244 0.2744	0.5739 0.2780	0.5429 0.6776	0.5000 >0.9999	0.6459 0.0601	0.6171 0.0613	0.6362 0.0737	0.6133 0.0817	0.6827 0.0517
IVS	0.5789 0.5066	0.5585 0.5033	0.5206 0.8551	0.6555 0.1085	0.6667 0.1007	0.6515 0.1076	0.5090 0.9084	0.6127 0.1125	0.6078 0.1454
BMI-for-age Z	-1.93 to -3.80	-1.43 to -1.93	1.0 to -1.43				1.0 to 1.5.	1.5 to 2.0	2.0 to 2.8
LDH[1]	0.5846 0.3360	0.5846 0.3360	0.5741 0.4214						
LV	0.5167 0.8744	0.6417 0.0586	0.5443 0.7114				0.6078 0.2443	0.5699 0.5800	0.5443 0.7114
LVIDs	0.5647 0.4492	0.5366 0.6099	0.5845 0.3591						

Note: The upper number in the cell denotes the AUC. the lower number denotes the p-value. the significance level. [1]CRE blood. LDH are taken from Table 5 for the BMI-for-age Z model. so they were estimated according to the BMI-for-age Z scale for the group higher than -1 and 1.

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