Relationships between the age and blood test results or body sizes in Noma horses

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The objective of this study is to analyze the relationships between the age and blood test results or body sizes in Noma horses by using the results of periodical health examination. Out of 45 hematological or physical items examined, statistically significant, but loose correlations were observed in 14 items. Red blood cell count, activities of aspartate aminotransferase, alkaline phosphatase, and creatinine kinase, concentrations of calcium and inorganic phosphorus decreased with aging. Conversely, mean corpuscular volume, mean corpuscular hemoglobin, lipase activity, γ-globulin and chloride concentrations, body height, chest circumference and cannon bone circumference increased with aging. The changes in a few items seemed unique to Noma horse. However, most age-related changes found in this study might be considered as a common trend in horse breeds rather than distinctive characteristic in Noma horse.

Key words: aging, blood test, body size, Noma horse
erected statistically significant when the $P$-values were less than 0.05. In several reports about the relationship between aging and blood profiles in horse [1–6, 10–13, 16], horses investigated were categorized in some age groups, and blood test results were compared among those groups by the significant difference examination. In small population such as Noma horse, however, there is difficulty to carry out comparative statistical analysis because of a few numbers of horses in each age group. So, in this study, correlation between age and the results of hematological and physical examination were analyzed.

The experimental protocols of this study were approved by the Animal Care and Use Committee of Okayama University of Science (approval number, 2019-13).

The mean values of hematological and serum biochemical test results and body sizes, and correlation coefficients with ages in days are shown in Table 1. Among 45 items inspected in the present study, there were statistically significant correlations with aging in 14 items. The items detected significantly positive correlation with aging were

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### Table 1. Means, standard deviations, and correlation coefficients of blood test results and body sizes in Noma horse

| Variable                 | n  | Mean   | SD    | $r$  | $P$   | Variable                 | n  | Mean   | SD    | $r$  | $P$   |
|--------------------------|----|--------|-------|------|-------|--------------------------|----|--------|-------|------|-------|
| RBC (10$^3$/μl)          | 49 | 773.4  | 106.4 | -0.581 | <0.001 | GLU (mg/dl)              | 49 | 111.6  | 17.9  | -0.280 | 0.051 |
| Hb (g/dl)                | 49 | 13.2   | 1.3   | -0.206 | 0.156  | T–BIL (mg/dl)             | 49 | 0.8    | 0.2   | 0.210  | 0.148 |
| Ht (%)                   | 49 | 37.8   | 3.7   | -0.250 | 0.083  | TG (mg/dl)                | 49 | 43.1   | 21.5  | -0.231 | 0.110 |
| MCV (fl)                 | 49 | 49.3   | 4.0   | 0.727  | <0.001 | T–CHO (mg/dl)             | 49 | 83.4   | 16.0  | 0.040  | 0.784 |
| MCH (pg)                 | 49 | 17.2   | 1.4   | 0.673  | <0.001 | TP (g/dl)                 | 49 | 7.3    | 0.7   | 0.104  | 0.475 |
| MCHC (g/dl)              | 49 | 34.8   | 0.4   | 0.100  | 0.494  | ALB (g/dl)                | 49 | 3.7    | 0.4   | -0.123 | 0.400 |
| WBC (10$^3$/μl)          | 49 | 70.7   | 13.4  | -0.271 | 0.059  | GLOB (g/dl)               | 49 | 3.7    | 0.6   | 0.161  | 0.268 |
| Neu (10$^3$/μl)          | 49 | 41.6   | 9.8   | -0.116 | 0.428  | A/G                       | 49 | 1.0    | 0.2   | -0.171 | 0.239 |
| Eos (/μl)                | 49 | 346.1  | 233.2 | -0.252 | 0.081  | α (g/dl)                  | 49 | 1.1    | 0.3   | -0.044 | 0.766 |
| Baso (/μl)               | 49 | 6.4    | 20.3  | -0.226 | 0.119  | β (g/dl)                  | 49 | 1.3    | 0.2   | 0.121  | 0.407 |
| Mono (/μl)               | 49 | 69.8   | 79.8  | -0.225 | 0.120  | γ (g/dl)                  | 49 | 1.4    | 0.3   | 0.314  | 0.028 |
| Lym (10$^3$/μl)          | 49 | 24.8   | 9.1   | -0.220 | 0.128  | Ca (mg/dl)                | 49 | 12.8   | 0.7   | -0.317 | 0.026 |
| PLT (10$^3$/μl)          | 49 | 13.4   | 3.4   | -0.130 | 0.374  | IP (mg/dl)                | 49 | 3.3    | 0.9   | -0.545 | <0.001 |
| LIPA (U/l)               | 49 | 17.2   | 4.8   | 0.318  | 0.026  | Mg (mg/dl)                | 49 | 2.2    | 0.2   | 0.123  | 0.400 |
| AST (U/l)                | 49 | 399.6  | 106.7 | -0.294 | 0.040  | Fe (μg/dl)                | 49 | 168.3  | 39.3  | 0.158  | 0.278 |
| LD (U/l)                 | 49 | 536.2  | 188.0 | -0.231 | 0.110  | TBA (μmol/l)              | 49 | 6.0    | 2.6   | 0.117  | 0.425 |
| ALP (U/l)                | 49 | 501.6  | 262.9 | -0.397 | 0.005  | Na (mEq/l)                | 49 | 140.8  | 2.8   | -0.099 | 0.498 |
| γ-GT (U/l)               | 49 | 18.2   | 6.4   | -0.013 | 0.030  | K (mEq/l)                 | 49 | 3.8    | 0.9   | -0.089 | 0.545 |
| CK (U/l)                 | 49 | 218.5  | 56.2  | -0.394 | 0.005  | Cl (mEq/l)                | 49 | 101.1  | 2.3   | 0.373  | 0.008 |
| AMY (U/l)                | 49 | 10.2   | 3.5   | 0.149  | 0.308  | BCS                       | 50 | 4.7    | 1.3   | -0.172 | 0.232 |
| BUN (mg/dl)              | 49 | 17.2   | 4.3   | 0.122  | 0.402  | BH (cm)                   | 50 | 108.6  | 6.4   | 0.291  | 0.040 |
| CRE (mg/dl)              | 49 | 1.0    | 0.2   | 0.237  | 0.101  | CC (cm)                   | 50 | 127.1  | 9.5   | 0.293  | 0.039 |
|                         |    |        |       |        |       | Cannon (cm)               | 50 | 13.6   | 0.9   | 0.417  | 0.003 |

The explanations of each abbreviation is as follows; number of horses tested (n), correlation coefficient ($r$), $P$-value ($P$), red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit value (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), neutrophil (Neu), eosinophil (Eos), basophil (Baso), monocyte (Mono), lymphocyte (Lym), platelet (PLT), lipase (LIPA), aspartate aminotransferase (AST), lactate dehydrogenase (LD), alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT), creatinine kinase (CK), amylase (AMY), blood urea nitrogen (BUN), creatinine (CRE), glucose (GLU), total bilirubin (T-BIL), triglyceride (TG), total cholesterol (TCHO), total protein (TP), albumin (ALB), globulin (GLOB), albumin/globulin ratio (A/G), α-, β-, and γ-globulins (α, β, and γ), calcium (Ca), inorganic phosphorus (IP), magnesium (Mg), iron (Fe), total bile acid (TBA), sodium (Na), potassium (K), chloride (Cl), nine-point scale body condition score (BCS), body height (BH), and chest circumferences (CC) and cannon bone circumferences (Cannon).
mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), lipase activity (LIPA), concentration of γ-globulin (γ) and chloride (Cl), body height (BH), chest circumferences (CC) and cannon bone circumferences (Cannon). Adversely, items having significantly negative correlations were red blood cell count (RBC), activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine kinase (CK), concentrations of calcium (Ca) and inorganic phosphorus (IP).

The changes of the hematological items in aged horse, i.e., lower RBC and higher MCV and MCH, showed a logarithm approximation and major change by 6-year-old (data not shown). Therefore, it might not be the characteristics in aged horse. Similar tendencies were also observed in Lipizzian horse [3], Lucitano horse [12] and donkey [16].

Mention to biochemical items, lower ALP, Ca and IP in aged horse were also reported in Thoroughbred [5] and donkey [16]. These decreases probably reflect decreased bone metabolism as animals become older [15]. In this study, the concentrations of ALP and IP showed major change by 6-year-old (data not shown). Significantly higher CK in younger horses compared to aged horses were also reported in Murinsulaner horse [1], but not in Thoroughbred [5]. Additionally, absence of age difference with AST is reported in several horse breeds [6, 11]. Significant positive correlations between AST and CK (r=0.561, P<0.001) were observed in this study. CK detected in plasma derive mainly from the skeletal muscle, and AST are from both hepatic cell and skeletal muscle [5]. So, these decreases in this study might reflect the decreases in momentum or muscle mass volumes with aging and are more outstanding compared to riding or working horses since Noma horses in this study are not used for these purposes. The age-related increase of γ also reported in racehorses [5]. Fraction of γ commonly increases in response to external antigenic stimuli resulting in a polyclonal gammopathy characterized by broad increases in the γ-fraction [7]. Our result with γ may indicate the increase of opportunity to be exposed to various antigen with aging. The increase of LIPA and Cl with aging were not reported in the previous studies. Silva et al. [11] reported that there were not significant differences with Cl between young and old horses of 48 breeds treated in their hospital.

Figure 2 shows relationships between the age and body sizes. The BH, CC and Cannon correlated positively with age. Bone growth developments of horse are thought to continue until 5-year-old [14]. In this study, for adult horses (>6 years), significant correlation was accepted only between age and the Cannon (n=41; r=0.347, P<0.05), not between age and the BH or CC. The result may indicate that only cannon bone growth continues after the general period of growth of frame in Noma horse.

The results of this study indicated some characteristics of the relation between hematological or physical examination and aging in Noma horse. However, any individual did not show obviously abnormal test results even in aged horses [13]. We considered that these age-related alterations might not be pathological, but rather physiological accompanied with aging. Furthermore, most age-related changes found in this study were also reported in other horse breeds. It might be considered as a common trend in horse breeds rather than distinctive characteristic in Noma horse. The results such as the decrease of AST and the increase of LIPA, Cl and Cannon seemed unique to Noma horse.

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**Fig. 2.** Correlations between the age and body sizes showing significant difference: n=50 in all items. The data of each physical item is depicted as the logarithm.
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