Blood Reference Intervals for Preterm Low-Birth-Weight Infants: A Multicenter Cohort Study in Japan

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¶ Membership of the Kyushu University High-Risk Neonatal Clinical Research Network, Japan is provided in the Acknowledgments.

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Abstract

Preterm low-birth-weight infants remain difficult to manage based on adequate laboratory tests. The aim of this study was to establish blood reference intervals (RIs) in those newborns who were admitted to and survived in the neonatal intensive care unit (NICU). A multicenter prospective study was conducted among all infants admitted to 11 affiliated NICUs from 2010 to 2013. The clinical information and laboratory data were registered in a network database designed for this study. The RIs for 26 items were derived using the parametric method after applying the latent abnormal values exclusion method. A total of 3189 infants were admitted during the study period; 246 were excluded due to a lack of blood sampling data, and 234 were excluded for chromosomal abnormalities (n = 108), congenital anomalies requiring treatment with surgical procedures (n = 76), and death or transfer to another hospital (n = 50). As a result, 2709 infants were enrolled in this study. Both the SDRBW and SDRGA were above 0.4 in the test results for total protein (TP), albumin (ALB), alanine aminotransferase (ALT), and red blood cells (RBC); their values increased in proportion to the BW and GA. We derived 26 blood RIs for infants who were admitted to NICUs. These RIs...
should help in the performance of proper clinical assessments and research in the field of perinatal-neonatal medicine.

Introduction

The prognosis of preterm low-birth-weight infants has improved dramatically with advances in perinatal medicine. The Neonatal Research Network of Japan revealed that more than 80% of infants delivered at 24 weeks of gestational age (GA) survived in neonatal intensive care units (NICUs) [1], and the survival rates of infants born at GA 22 and 23 weeks were also improved compared with those in previous studies [2]. However, the treatment of these vulnerable newborns and the associated clinical research remain great challenges. It is essential to properly assess these infants based on adequate physiological data [3] as well as laboratory tests.

Due to the nature of the physiological growth and development of infants and children, many efforts have been made to establish pediatric reference intervals (RIs) for routinely-measured laboratory parameters [4] [5]. Christensen et al. published RIs for the complete blood cell (CBC) counts in term and preterm infants using the large database of a health care system [6] [7] [8] [9]. The Canadian Laboratory Initiative on Pediatric Reference Interval Database established RIs for blood chemistry data in healthy and multiethic child populations [10] [5]. In contrast, few studies have been published regarding the RIs of blood chemistry elements for preterm low-birth-weight infants [11] [12] [13].

The “Kyushu University High-Risk Neonatal Clinical Research Network” Project is a collaborative study conducted among NICUs across the northern part of Kyushu Island in Japan from April 2010 to March 2013. For this project, a prospective observational study was performed in the 11 affiliated hospitals in order to establish blood RIs in preterm low-birth-weight infants within 24 h after birth who were admitted to multiple perinatal care centers and who survived until hospital discharge.

Materials and Methods

Multicenter Prospective Study

All infants admitted to any of the 11 affiliated NICUs on the first day of life were enrolled in this study, with the following exclusion criteria: chromosomal abnormalities, congenital anomalies requiring surgical procedures during the neonatal period, and death or transfer to other hospitals prior to discharge. Data acquisition was carried out using a web-based electronic medical software program that stores laboratory data and clinical information (Hitachi Solutions, Ltd., Tokyo, Japan). The data were classified into three subgroups based on either by BW or GA according to the classification of the World Health Organization (WHO) for neonates. The study protocol was approved by the Institutional Review Board (#22–131; Kyushu University Hospital) at each institution and registered as a prospective observational study with the University Hospital Medical Information Network clinical trial registration system in Japan (UMIN000008763) in April 2010. Written informed consent was obtained from all of the caretakers of the patients prior to their enrollment in this study.

Target Test Items and Standardization of Measurements

The Japan Society of Clinical Chemistry established common RIs for use nationwide in Japan for 40 commonly tested laboratory tests [14]. Annual external quality controls have been done
for the major analytes among the affiliated medical facilities in the northern region of Kyushu Island[15], and we confirmed the standardized status of all the assays[16]. The CBC count and differential white blood cell counts were measured using automated Beckman Coulter Hematology Analyzers (Beckman Coulter Inc., FL, USA). The following 16 biochemical and 10 hematological test items were chosen as analytes: total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), total bilirubin (T-BIL), direct bilirubin (D-BIL), sodium (Na), potassium (K), chloride (CL), calcium (Ca), C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine kinase (CK), white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT), neutrophils (NEUT), lymphocytes (LYMP), monocytes (MONO), eosinophils (EOS) and basophils (BASO).

Statistical Analysis

The following items, which may be affected by a pathological state, were excluded depending on the international classification of diseases-10 (ICD-10) code: LDH, AST, and CK in infants with a disease code of P20-29; respiratory and cardiovascular disorders specific to the perinatal period and CRP in infants with P35-39; infections specific to the perinatal period, K, and T-BIL in infants with P50-61; and hemorrhagic and hematological disorders specific to fetuses or newborns. In order to exclude inappropriate infants with multiple abnormal results, a multivariate iterative method called latent abnormal values exclusion (LAVE) [14][15][16] was applied for simultaneous derivation of RIs for multiple test items. In this study, the LAVE method was used for the values of WBC, HGB, HCT, TP, BUN, CK, K, LDH, ALT, and CRP with eight iterations and an allowance of up to one result outside the RI and up to one missing result in the reference test items.

The parametric method was used for computing the RIs after transforming the distribution of the reference values into a Gaussian form using a modified Box-Cox transformation [14]. The 90% confidence intervals (CIs) for the upper (UL) and lower limits (LL) of the RIs were estimated by the bootstrap method to avoid any abnormal results in the reference test [17, 18]. The need to partition the reference values by sex, GA, and BW was judged based on the SD ratio (SDR) introduced by Ichihara [14]; the SDR for sex (SDRSEX) is expressed as the SD representing the sex difference divided by the SD of the RI (SDRI, or 1/4th of RI). Similarly, the SDRs for GA and BW (SDRGA, SDRBW) were computed as a ratio of the SD representing the between-GA and between-BW subgroup differences divided by the SDRI, respectively. We adopted an SDR cutoff value of 0.4 by consensus among the collaborators [17, 19, 20].

Results

Outline of the Prospective Study

Fig 1 shows an outline for the recruitment of preterm low-birth-weight infants in this prospective study. A total of 3,189 infants were hospitalized on the first day of life at the 11 NICUs between April 2010 and March 2013; 246 did not undergo laboratory testing on admission, 210 were recognized to be healthy and did not require blood testing, 9 were transferred to other hospitals, and 27 died within 24 hours after birth. We also excluded 234 infants who received a diagnosis of congenital abnormalities (n = 210) or congenital abnormalities requiring surgical procedures within 28 days after birth (n = 76) or who died in the hospital or were transferred to other facilities (n = 50). Therefore, the final study group for analyzing the blood RIs comprised 2,709 infants who did not meet the exclusion criteria and survived until hospital discharge.
ICD-10

Table 1 shows the number of subjects in the final group classified based on the ICD-10 code. We diagnosed 1,555 low-birth-weight infants (91.6%) (Total < 2,500 g) and 1,340 preterm birth infants (89.9%) (total < 37 w) with P05-08: disorders related to the length of gestation and fetal growth. S1 Table displays the ICD-10 diagnostic information for the excluded infants.

GA- and BW-specific Blood RIs

Table 2 displays the GA-specific RIs of hematology and blood chemistry (international units). The items with SDRs of more than 0.4 were subgrouped based on the GA classification. The SDR\textsubscript{GA} values of TP, ALB, CRE, Na, ALT, WBC, RBC, NEUT and MONO were significant. The BW-specific RIs for low-birth-weight infants (international units) are shown in Table 3. The SDR\textsubscript{BW} of TP, ALB, ALT, RBCs, and EOS were significant. The values of TP, ALB, ALT and RBC were significant in both SDR\textsubscript{GA} and SDR\textsubscript{BW}, with values of more than 0.4. These
levels increased in proportion to the BW and GA (Fig 2). S2 and S3 Tables show the RIs converted from international to conventional units.

**Discussion**

The Kyushu University High-Risk Neonatal Clinical Research Network Project recently established a university initiative to accumulate information for all infants admitted to the affiliated NICUs using a web-based electronic medical software program. As part of this initiative, our study’s main purpose was to establish RIs for hematology and blood chemistry analytes in preterm low-birth-weight infants who were admitted to perinatal-neonatal care centers in Japan and who survived until discharge.

We were able to obtain a sufficient sample size (2709 infants) during the three-year study period. The practically attainable target sample size for each analyte was set at a minimum of 250 or more, which is greater than twice the minimum number (120 or more) [19]. We excluded outliers based on the clinical diagnosis (ICD-10) and the results of the multivariate

**Table 1. Number of preterm low-birth-weight infants classified in the ICD-10 (n = 2709).**

| ICD-10 codes | Total <2500g | Total <37w | Classified by BW | Classified by GA |
|--------------|-------------|------------|------------------|------------------|
|              | <1000g | 1000-1500g | 1500-2500g | <28w | 28-32w | 32-37w |
| P00-P04 Fetus and newborn affected by maternal factors and by complications of pregnancy, labour and delivery | 5 | 0 | 0 | 9 | 0 | 0 |
| P05-P08 Disorders related to length of gestation and fetal growth | 1555 | 148 | 225 | 1182 | 1340 | 124 | 215 | 1001 |
| P10-P15 Birth trauma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P20-P29 Respiratory and cardiovascular disorders specific to the perinatal period | 74 | 0 | 2 | 72 | 88 | 0 | 1 | 87 |
| P35-P39 Infections specific to the perinatal period | 8 | 0 | 0 | 8 | 6 | 0 | 0 | 6 |
| P50-P51 Haemorrhagic and haematological disorders of fetus and newborn | 14 | 1 | 0 | 13 | 6 | 1 | 0 | 5 |
| P70-P74 Transitory endocrine and metabolic disorders specific to fetus and newborn | 34 | 0 | 3 | 31 | 31 | 0 | 1 | 30 |
| P75-P78 Digestive system disorders of fetus and newborn | 3 | 0 | 0 | 3 | 5 | 0 | 0 | 5 |
| P80-P83 Conditions involving the integument and temperature regulation of fetus and newborn | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P90-P96 Other disorders originating in the perinatal period | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| Q00-Q07 Congenital malformations of the nervous system | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q10-Q18 Congenital malformations of eye, ear, face and neck | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| Q20-Q28 Congenital malformations of the circulatory system | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q30-Q34 Congenital malformations of the respiratory system | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q35-Q37 Cleft lip and cleft palate | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Q38-Q45 Other congenital malformations of the digestive system | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 2 |
| Q50-Q56 Congenital malformations of genital organs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q60-Q64 Congenital malformations of the urinary system | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q65-Q79 Congenital malformations and deformations of the musculoskeletal system | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q80-Q89 Other congenital malformations | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q90-Q99 Chromosomal abnormalities, not elsewhere classified | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **1697** | **150** | **230** | **1317** | **1489** | **126** | **217** | **1146** |

BW; birth weight, GA; gestational age, According with the World Health Organization classification of BW and GA, the data were collected for sub-stratified by six BW and GA groups: low-birth-weight infant as a BW of <2500g, very low-birth-weight infant as <1500g, extremely low-birth-weight infant as <1000g, and moderate to late preterm infant as GA of <37w, very preterm infant as <32w, extremely preterm infant as <28w, respectively.

doi:10.1371/journal.pone.0161439.t001
Table 2. GA-specific RIs of blood chemistry and hematology for preterm infants (International Unit).

| Analyte | SI unit | SDR<sub>GA</sub> | LL%CI | RI | UL%CI | LL%CI | RI | UL%CI | LL%CI | RI | UL%CI | LL%CI | RI | UL%CI |
|---------|---------|------------------|-------|----|-------|-------|----|-------|-------|----|-------|-------|----|-------|
| TP      | g/L     | 1.02             |       | 113 | 25    | 30    | 27  | 48    | 44    | 52  | 194   | 33    | 35  | 34    |
| ALB     | g/L     | 0.05             |       | 96   | 19    | 22    | 20  | 32    | 30    | 34  | 161   | 25    | 26  | 25    |
| BUN     | μmol/L  | 0.14             | 1476  | 1.38 | 1.57  | 1.44  | 5.98| 5.65  | 6.41  | 3.61| 1164  | 4.01  | 4.1 | 4.01  |
| CRE     | μmol/L  | 0.44             |       | 112 | 24.8  | 29.2  | 27.4| 79.7  | 72.6  | 86.7| 192   | 24.8  | 31.0| 30.1  |
| T-BIL   | μmol/L  | 0.36             | 1432  | 20.5 | 21.9  | 21.0  | 57.8| 56.6  | 61.2  | 54.1| 1050  | 133   | 134| 134   |
| D-BIL   | μmol/L  | 0.00             | 868   | 5.6  | 5.8   | 5.8   | 19.2| 18.3  | 20.2  | 19.2| 1050  | 133   | 134| 134   |
| Na      | mmol/L  | 0.40             |       | 944  | 1288  | 130   | 129| 142   | 141   | 144| 162   | 131   | 133| 131   |
| K       | mmol/L  | 0.00             | 1272  | 3.6  | 3.7   | 3.7   | 6.1 | 6.0   | 6.2   | 6.1 | 162   | 131   | 133| 131   |
| Cl      | mmol/L  | 0.00             | 1314  | 100  | 101   | 111   | 111 | 111   | 111   | 111| 1050  | 133   | 134| 134   |
| Ca      | mmol/L  | 0.18             | 1466  | 2.0  | 2.0   | 2.0   | 2.7 | 2.6   | 2.7   | 2.7| 1050  | 133   | 134| 134   |
| CRP     | μg/L    | 0.14             | 1356  | 0.00 | 0.00  | 0.00  | 0.80| 0.20  | 0.72  | 0.72| 1050  | 133   | 134| 134   |
| AST     | IU/L    | 0.28             | 1281  | 15   | 16    | 16    | 70  | 65    | 78    | 78 | 1050  | 133   | 134| 134   |
| ALT     | IU/L    | 0.66             |       | 105  | 1     | 1     | 1   | 6     | 5     | 9  | 193   | 1     | 1  | 1     |
| LDH     | IU/L    | 0.11             | 1297  | 238  | 263   | 245   | 787 | 742   | 968   | 968| 1050  | 133   | 134| 134   |
| ALP     | IU/L    | 0.16             | 462   | 351  | 397   | 372   | 1082| 1152  | 1152  | 1152| 1050  | 133   | 134| 134   |
| CK      | IU/L    | 0.44             |       | 88   | 22    | 60    | 45  | 916   | 583   | 1263| 173   | 38    | 76  | 53    |
| WBC     | 10⁹/L   | 0.57             |       | 102  | 2.55  | 3.87  | 3.04| 31.0  | 22.5  | 39.2| 179   | 2.68  | 4.07| 3.40  |
| RBC     | 10⁹/L   | 0.59             |       | 103  | 2.63  | 2.98  | 2.76| 4.65  | 4.51  | 4.82| 178   | 3.20  | 3.41| 3.28  |
| HGB     | g/L     | 0.33             | 1406  | 125  | 129   | 127   | 203 | 201   | 206   | 206| 1050  | 133   | 134| 134   |
| HCT     | /L      | 0.31             | 1408  | 0.37 | 0.38  | 0.38  | 0.60| 0.60  | 0.61  | 0.61| 1050  | 133   | 134| 134   |
| PLT     | 10⁹/L   | 0.20             | 1344  | 107  | 120   | 114   | 375 | 367   | 375   | 375| 1050  | 133   | 134| 134   |
| NEUT    | 10⁹/L   | 0.79             |       | 46   | 0.17  | 0.81  | 0.64| 17.0  | 11.7  | 27.6| 91    | 0.15  | 0.68| 0.37  |
| LYMP    | 10⁹/L   | 0.30             | 754   | 1.88 | 2.36  | 2.06  | 9.72| 9.21  | 10.3  | 10.3| 1050  | 133   | 134| 134   |
| MONO    | 10⁹/L   | 0.64             |       | 52   | 30    | 152   | 57  | 1623  | 1270 | 2185| 118   | 29    | 110| 64    |
| EOS     | 10⁹/L   | 0.32             | 745   | 6    | 36    | 8     | 741 | 693   | 797   | 797| 1050  | 133   | 134| 134   |
| BASO    | 10⁹/L   | 0.10             | 748   | 1    | 2     | 2     | 270 | 241   | 300   | 300| 1050  | 133   | 134| 134   |

TP; total protein, ALB; albumin, BUN; blood urea nitrogen, CRE; creatinine, T-BIL; total bilirubin, D-BIL; direct bilirubin, Na; sodium, K; potassium, Cl; chloride, Ca; calcium, CRP; C-reactive protein, AST; aspartate aminotransferase, ALT; alanine aminotransferase, LDH; lactate dehydrogenase, ALP; alkaline phosphatase, CK; creatine kinase, WBC; white blood cell, RBC; red blood cell, HGB; hemoglobin, HCT; hematocrit, PLT; platelet, NEUT; neutrophil, LYMP; lymphocyte, MONO; monocyte, EOS; eosinophil, BASO; basophil, GA; gestational age, RI; reference interval, LL; lower limit of the RI, UL; upper limit of the RI, CI; confidential interval (90%) of LLs and ULs were estimated by the bootstrap method. The results were excluded data of LDH, AST and CK in the infants with P20-29; Respiratory and cardiovascular disorders specific to the perinatal period, CRP with P35-39; Infections specific to the perinatal period, and K and T-Bil with P50-61; Hemorrhagic and homologous disorders specific to fetus and newborn, respectively. A multivariate iterative method called latent abnormal value exclusion (LAVE) was applied to nine analytes (TP, BUN, K, LDH, ALT, WBC, CRP, HGB and HCT) which deemed to be adversely affected by hemolysis and inflammation. By use of 3-level nested ANOVA, the influence of GA on test results was expressed in terms of standard deviation (SD) ratio (SDR), as SDR<sub>GA</sub>. SDR> = 0.4 were used as a criteria for the need of partition by the factor.
Table 3. BW-specific RIs of blood chemistry and hematology for low-birth-weight infants (International Unit)

| Analyte | SI unit | SDR_{BW} | Total <2500g | Classified by BW | 1000-1500g | 1500-2500g |
|---------|---------|----------|--------------|-----------------|------------|------------|
| TP | g/L | 0.94 | 132 26 31 29 51 48 57 202 33 35 34 60 58 63 1178 40 41 40 44 63 65 |
| ALB | g/L | 0.89 | 112 19 22 21 35 33 37 169 25 25 25 38 37 39 1010 28 28 28 40 40 41 |
| BUN | mmol/L | 0.00 1.38 1.59 1.46 6.12 5.78 6.51 |
| CRE | μmol/L | 0.32 31.0 35.4 31.9 85.0 81.4 92.0 |
| T-BIL | μmol/L | 0.11 | 1474 20.5 22.1 21.2 60.7 58.0 64.6 |
| D-BIL | μmol/L | 0.00 | 917 5.5 5.8 5.6 20.0 19.0 21.0 |
| Na | mmol/L | 0.38 | 1315 132 133 133 143 142 143 |
| K | mmol/L | 0.10 | 1519 2.0 2.0 2.0 2.7 2.6 2.7 |
| CL | mmol/L | 0.13 | 1315 100 101 101 111 111 112 |
| Ca | mmol/L | 0.10 | 1519 2.0 2.0 2.0 2.7 2.6 2.7 |
| CRP | μg/L | 0.00 | 1521 0.0 0.0 0.0 5.1 0.3 7.7 |
| AST | IU/L | 0.00 | 1268 15 16 15 74 70 82 |
| ALT | IU/L | 0.09 | 1281 235 261 243 835 767 984 |
| LDH | IU/L | 0.00 | 1268 15 16 15 74 70 82 |
| ALP | IU/L | 0.00 | 463 352 393 372 1097 1051 1181 |
| CK | IU/L | 0.34 | 1258 58 73 64 685 641 744 |
| WBC | 10^9/L | 0.22 | 1473 4.13 4.91 4.42 24.0 23.0 25.6 |
| RBC | 10^9/L | 0.09 | 1450 125 129 127 204 203 206 |
| HGB | g/L | 0.36 | 1454 0.37 0.39 0.38 0.61 0.60 0.61 |
| HCT | /L | 0.29 | 1454 0.37 0.39 0.38 0.61 0.60 0.61 |
| PLT | 10^9/L | 0.21 | 1392 108 121 108 375 365 383 |
| NEUT | 10^9/L | 0.27 | 748 0.74 1.07 0.90 17.5 15.9 19.1 |
| LYMP | 10^9/L | 0.00 | 877 1.67 2.15 1.54 9.37 8.93 9.78 |
| MONO | 10^9/L | 0.21 | 875 116 164 143 159 159 190 |
| EOS | 10^4/L | 0.42 | 1 | 24 5 411 289 552 112 0 6 0 555 462 755 596 45 73 59 867 778 932 |
| BASO | 10^6/L | 0.09 | 823 1 3 2 284 249 313 |

TP; total protein, ALB; albumin, BUN; blood urea nitrogen, CRE; creatinine, T-BIL; total bilirubin, D-BIL; direct bilirubin, Na; sodium, K; potassium, CL; chlorine, Ca; calcium, CRP; C-reactive protein, AST; aspartate aminotransferase, ALT; alanine aminotransferase, LDH; lactate dehydrogenase, ALP; alkaline phosphatase, CK; creatine kinase, WBC; white blood cell, RBC; red blood cell, HGB; hemoglobin, HCT; hematocrit, PLT; platelet, NEUT; neutrophil, LYMP; lymphocyte, MONO; monocyte, EOS; eosinophil, BASO; basophil, GA; gestational age, RI; reference interval, LL; lower limit of the RI, UL; upper limit of the RI, CI; confidential interval (90%) of LLs and ULs were estimated by the bootstrap method. The results were excluded data of LDH, AST and CK in the infants with P20-29; Respiratory and cardiovascular disorders specific to the perinatal period, CRP with P35-39; Infections specific to the perinatal period, and K and T-Bil with P50-61; Hemorrhagic and homological disorders specific to fetus and newborn, respectively. A multivariate iterative method called latent abnormal value exclusion (LAVE) was applied to nine analytes (TP, BUN, K, LDH, ALT, WBC, CRP, HGB and HCT) which deemed to be adversely affected by hemolysis and inflammation. By use of 3-level nested ANOVA, the influence of BW on test results was expressed in terms of standard deviation (SD) ratio (SDR), as SDR_{BW}. SDR > 0.4 were used as a criteria for the need of partition by the factor.

doi:10.1371/journal.pone.0161439.t003
iterative method (LAVE) [19]. The LAVE features the simultaneous setting of RIs for multiple test items that are mutually related and the rigid exclusion of individuals with abnormal values for other test items [20]. As a result, the reference individuals were “considered to be normal” preterm low-birth-weight infants based on the ICD-10 code (Table 1).

The Ichihara method utilizes information for the SDR attributable to each source of variation and can be applied in situations in which more than two subgroups are categorized according to the factors [15] [18]. In our study, we presented each RI classified into six subgroups by the BW and GA according to the SDR_{BW} and SDR_{GA}, respectively. The SDR_{SEX} of each item were zero for all analytes for the age groups (data not shown). Therefore, sex-specific RIs were not presented in this study.

In our study, the following tended to increase in proportion to both the GA and BW: TP, Alb, and ALT (Tables 2 and 3 and Fig 2). The TP is made up of Alb and globulin. Alb is synthesized in the liver, and a low serum Alb may result from immaturity of the liver function. ALT is an important transaminase enzyme in various tissue, especially the liver; therefore, the blood ALT level is used clinically as a biomarker for the liver function. In small-for-gestational-age infants, the AST and ALT serum activities were correlated with BW and GA [21]. Our data

![Fig 2. Scatter plots of the items that were significant in both the SDR_{GA} and SDR_{BW}. The horizontal axis shows the birth weight (BW). The vertical axis shows the measured values of A. Total protein (g/dL), B. Albumin (g/dL), C. Alanine aminotransferase (ALT) (IU/L), and D. Red blood cells (10^6/μL). The data were classified into three subgroups based on gestational age (GA): GA of 32–37 weeks, cross marks (×); 28–32 weeks, open circles (○); 22–28 weeks, filled circles (●). doi:10.1371/journal.pone.0161439.g002](image-url)
confirmed the parallel upward trend in these values as the organ function matured. In contrast, extremely premature infants had high plasma enzyme activities compared to babies at a later corrected GA [22], possibly due to suffering more severe illness immediately after birth.

Significant SDR_{GA} and SDR_{BW} values (>0.4) were observed for WBC, RBC, NEUT, and MONO (Table 2); and RBC and EO (Table 3), respectively. When the test results of those analytes were compared among the groups, the RBC count was found to have a tendency to increase in proportion to both the BW and GA (Fig 2). The RIs of HCT and HGB in extremely preterm patients were reported to be lower than those in later preterm and term infants [23]. Several reports have shown the same gradual upward tendency in hematological data [9][23]. The PLT counts increased for GAs of 22 to 42 weeks using a huge data system [8]. In contrast, an abnormal lymphocyte count at birth is associated with adverse outcomes, including early-onset sepsis, intraventricular hemorrhaging and retinopathy of prematurity [24]. The onset of neutropenia in the first days of life is sometimes noted in SGA infants or those born to mothers with persistent maternal hypertension or early-onset bacterial infection [25]. These reports suggest that the GA and BW, as well as potentially pathogenic maternal and neonatal variables, should be considered when developing RIs.

We recognize various limitations and pitfalls that should be considered when applying these RIs in practice. First, preterm or low-birth-weight babies are considered to be in a clinically pathological or unhealthy state, and many require medical management. Therefore, “normal range” is not a suitable term for the blood chemistry and hematology data for these infants. We therefore used the term “reference interval” in this project and discarded data confirmed to be unacceptable based on the ICD-10 code and LAVE method. A second limitation is that the source of blood specimens (capillary, venous, or arterial) was not taken into account. Some hematological and chemical test values are somewhat higher in capillary samples than in venous or arterial samples. The third limitation is that we did not analyze the trends in the values after birth [23]. The values of analytes may change after several postnatal days depending on the clinical course of the infant.

Conclusions
Our project provides 26 blood RIs in preterm low-birth-weight infants requiring neonatal intensive care in Japan. These RIs should help researchers in the field of perinatal-neonatal medicine perform proper assessments in routine clinical work and research. Further evaluations are needed to determine whether these RIs are representative of the physiological data for those infants.

Supporting Information
S1 Table. The ICD-10 of the excluded infants
(DOCX)

S2 Table. GA-specific RIs of blood chemistry and hematology for preterm infants (Conventional Unit)
(DOCX)

S3 Table. BW-specific RIs of blood chemistry and hematology for low birth weight infants (Conventional Unit)
(DOCX)

Acknowledgments
Institutions and representative physicians enrolled in the database for the Kyushu University High-Risk Neonatal Clinical Research Network Project include: Fukuoka Children’s Hospital
and Medical Center for Infectious Diseases: Yasushi Takahata; National Hospital Organization Kyushu Medical Center: Kazuo Sato; Japanese Red Cross Fukuoka Hospital: Shunji Hikino; General Hospital Hamanomachi: Megumi Takemoto; Japan Community Health care Organization Kyushu Hospital: Junko Yamamoto; Kitakyushu Municipal Medical Center: Naoko Matsumoto; National Hospital Organization Kokura Medical Center: Hironori Yamashita; National Hospital Organization Beppu Medical Center: Hiroshi Koga; Oita Prefectural Hospital: Koichi Iida; Yamaguchi Red Cross Hospital: Shinichi Terachi, and Kyushu University Hospital: Shouji Tokunaga, Takeru Abe, Yoko Fukuda. We are indebted a great deal to Mr Shogo Kimura of Yamaguchi University Graduate School of Medicine who devoted to perform the statistical analyses and to produce graphs presented in this paper.

**Author Contributions**

Conceived and designed the experiments: MO YM TK HI NN KIh SO TH.

Performed the experiments: DK.

Analyzed the data: KIc.

Contributed reagents/materials/analysis tools: MO YM TK HI.

Wrote the paper: MO YM.

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