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Journal

Veterinary Clinical Pathology, 45(1)

ISSN

0275-6382

Authors

White, Jessica R
Gong, Huiyu
Colaizy, Tarah T
et al.

Publication Date

2016-03-01

DOI

10.1111/vcp.12314

Peer reviewed

HHS Public Access

Author manuscript

Vet Clin Pathol. Author manuscript; available in PMC 2017 March 01.

Published in final edited form as:

Vet Clin Pathol. 2016 March ; 45(1): 87–95. doi:10.1111/vcp.12314.

Evaluation of hematologic composition in newborn C57/BL6 mice up to day 35

Jessica R White[1], **Huiyu Gong**[1], **Tarah Colaizy**[1], **Jessica G Moreland**[1], **Heather Flaherty**[2], and **Steven J McElroy**[1]

[1]Pediatrics, University of Iowa, Iowa City, IA, USA

[2]Veterinary Pathology, Iowa State University, Ames, IA, USA

Abstract

Background—Hematologic variables are often analyzed in animal analogues during the investigation of complex disease etiologies such as necrotizing enterocolitis. However, reference intervals (RI) can vary depending on animal strain, age, and sampling site. Reference intervals have been published for adult C57BL/6J mice, but not newborn C57BL/6J mice.

Objectives—The purpose of the present study was to determine hematologic RI in newborn C57BL/6J mice up to day 35.

Methods—C57BL/6J mice founders from The Jackson Laboratory were bred at the University of Iowa. Blood samples were obtained via facial vein sampling at postnatal days 0 (p0), p7, p14, p21, p28, or young adulthood (p35). CBCs were determined with the Sysmex XT-2000iV analyzer within 30 minutes of blood collection at a 1:10 dilution. Statistics were determined using nonparametric methods following ASVCP guidelines.

Results—Hematologic RI were determined for each of the 6 groups (n=247, n≥39 per group). Significantly higher values for HGB, RBC, and PLT counts were observed with advancing developmental age. Total WBC counts remained relatively stable during the first 35 days of life. However, WBC differential counts were dominated by neutrophils and lymphocytes in the younger mice, with a trend towards a lymphocytic leukogram on day 35.

Conclusions—These results illustrate the dynamic changes in hematologic variables during murine development after birth. Utilization of age-specific RI is advised when evaluating data derived from experimental perinatal mouse models.

Keywords

blood; neonates; ontogeny; models of human disease; necrotizing enterocolitis

Correspondence: Steven J McElroy, University of Iowa Pediatrics, 1270A CBRB, 285 Newton Road, Iowa City, IA 52242, steven-mcelroy@uiowa.edu.

Disclosure: The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

Introduction

The C57BL/6J mouse is one of the most commonly used inbred murine background strains to generate transgenic models of various human diseases, including those found in neonates and children. These studies often include evaluation of changes in cell and protein concentrations to detect dysfunctions within a system that could contribute to the disease process. Our laboratory uses mouse models to examine the immature gastrointestinal system, and specifically to study necrotizing enterocolitis (NEC), a devastating disease of premature human newborns.[1-3] Despite leading to the death of close to 500 infants born yearly in the United States[4,5], NEC remains incompletely understood. While it is hypothesized that microbial-induced inflammation in an immature intestinal tract is key to pathogenesis of this disease, several recent studies have noted an association between infants who develop NEC and those who had recently received blood product transfusions. [6,7] The hematologic system is critical for many mammalian functions such as transport of oxygen and carbon dioxide, delivery of nutrients to the tissues, and delivery of immune defense components to sites of damage or infection. Thus, understanding the hematologic system is integral to understanding NEC and many other diseases.

Previous studies have suggested differences in hematologic variables during the postnatal period compared to adulthood in multiple different species.[8-11] While hematologic reference intervals (RI) are available for adult mice[12-14] and neonatal mice of select strains (CF-1 mice[15]), such RI have not been described in neonatal C57BL/6J mice, nor have they been adequately defined in any strain during murine development from neonate to early adulthood. The purpose of the present study was to define hematologic RI in healthy newborn C57BL/6J mice from birth to postnatal day 35.

The animal sample population for this study included C57BL/6J mice born from founders from The Jackson Laboratory. Mice were raised at The University of Iowa under standard housing conditions. The Institutional Animal Care and Use Committee at The University of Iowa approved all animal experimental protocols, and protocols complied with institutional guidelines for research on animals. Mice were nursed until p21 and then given standard gruel until day 35. Healthy male and female mice were evaluated at 6 defined developmental stages: postnatal day 0 (p0), p7, p14, p21, p28, and p35/adult. All assessments were performed at the same time of day.

All mice were weighed prior to blood sampling. Facial vein sampling without anesthesia was performed with a 3 mm Goldenrod animal lancet (Braintree Scientific, Braintree MA, USA). Blood was collected into 100 μl EDTA microvette tubes (Sarstedt, Numbrecht Germany) and gently mixed to avoid platelet activation. Twelve microliters of whole blood were then transferred to a microcentrifuge tube containing 108 μl CellPak (Sysmex America, Lincolnshire IL, USA), as recommended by the manufacturer.

The Sysmex XT-2000iV (Sysmex America, Lincolnshire IL, USA) requires 85 μl for a full standard hematology profile, however the available sample volume from P0 animals was only 20-30 μl. Therefore, samples were diluted 10-fold, and not 5-fold as recommended by Sysmex for the capillary mode. To minimize variability, all samples, including those from

older mice, were diluted 10-fold, inspite of larger available sample volumes. To determine a potential dilution effect on precision and accuracy, split samples collected from mice on day 7 were diluted at 1:5 and 1:10, and the data compared by ANOVA. All experiments were performed in triplicate.

Samples were placed on ice until analysis within approximately 30 min of sampling, and analysis was performed using a Sysmex XT-200iV (Sysmex America, Lincolnshire IL, USA) analyzer in manual capillary mode. The measurement ranges as determined by Sysmex for hematology variables are provided in Table 1. Measured variables included RBC count, HGB concentration, MCV, MCH, MCHC, absolute and relative reticulocyte counts, PLT concentration, mean platelet volume (MPV), and total and differential WBC counts.

Reference intervals were determined according to ASVCP guidelines.[16] SAS 9.3.2 was used to calculate the RI (2.5^{th}-97.5^{th} percentiles) and 90% confidence intervals (CI). Normality was assessed using Shapiro-Wilk test, Kolmogorov-Smirnov test, and Anderson-Darling test, and visually by histogram examination. Winsorized means and 90% CI intervals were calculated, as this robust estimating method is relatively insensitive to outliers. Nonparametric tests were used to compare differences at individual time points (Kruskal-Wallis, PRISM 6.0).

There was no effect on accuracy and precision of hematologic variables due to dilution of blood from 1:5 to 1:10. Figure 1 shows the comparison of results obtained after 1:5 and 1:10 dilution of blood from mice on day 7.

The mean, median, SD, reference limits, RI and their 90% CIs are reported in Tables 2-7, frequency histograms are shown in Figure 2, and the course over time of select variables is shown in Figure 3. An overall increase in RBC counts with advancing age was noted, with significant differences between p7 and p14 (P=.0005), and between p14 and p21 (P=.0332). There was a statistically significant initial HGB decline during the first week of life (P= .0095) followed by a surge between p14 and p21 (P=.0005), and a steady increase between p21 and p28 (P=.0029). There was a significant MCV decrease between p7 and p14 (P<.0001); likewise, MCH also declined significantly between p7 and p14 (P<.0001), and p14 and p21 (P<.0054), before increasing significantly between p21 and p28 (P<.0003). MCHC also declined significantly from p7 to p14 (P<.0015), and p14 and p21 (P<.0001), before rising between p21 and p28 (P<.0001). Absolute reticulocyte counts also increased significantly between p0 and p7, and p14 and p21, and then declined to adult values by p28 (P<.0001 for each interval). In general, PLT counts increased with increasing age, with a significant increase between p7 and p14 (P<.0001), accompanied by a significant decrease of MPV (P<.0001).

The WBC counts were relatively stable over the first 2 weeks of life, but increased significantly between p14 and p21 (P<.0001), and p28 and p35 (P<.0017). The WBC differential count at birth was characterized by equal proportions of neutrophils and lymphocytes. With advancing age, the differential count was increasingly dominated by lymphocytes. Eosinophils usually accounted for <3.5% of the total WBC count at each

developmental stage, with a significant increase noted between p0 and p7 (*P*=.0003) and a decrease between p14 and p21 (*P*=.027). Basophils were present at low numbers at birth (1.5% of total WBC count) and became negligible by p14 (Figure 4).

Hematologic variables are key indicators of health and disease, but only provided that valid species, sex and age-specific RI from a healthy population allow an adequate assessment of the changes observed in clinical disease or research studies. Our data from newborn C57BL/6J mice illustrate significant age-dependent changes and trends in hematologic variables between birth and adulthood, while RBC, HGB, PLT, and WBC values at p35 were comparable to the RI provided by The Jackson Laboratory for adult C57BL/6J mice. [20] Specifically, we noted trends in RBC, HGB, MCV, PLT, and absolute reticulocyte counts with advancing age and weight, which reached statistical significance for HGB, RBC, and PLT increases over the course of development, with the exception of a brief drop in HGB and PLT after the first week of life (p7).

Laboratory data including hematologic variables can vary depending on the methods and instruments used for measurement. Specifically, due to the very low sample volumes collected from newborn mice we were forced to use a dilution of 1:10 instead the 1:5 recommended for the Sysmex XT-2000iv in capillary mode. However, a comparison of samples diluted at 1:10 with samples diluted at 1:5 did not reveal significant differences. Additionally, variables with low numbers such as eosinophils and basophils had relatively small error bars, suggesting an absence of major variations due to the higher dilution. While a full method comparison validation study would be desirable to assure the lack of a bias in connection with diluted samples, it would be challenging to recruit a sufficient number of newborn mice to perform just such an exercise. Nevertheless, the lack of standard validation represents a small limitation of this study.

In our study we found that the differential WBC counts changed towards a lymphocytic leukogram with advancing age. We hypothesize that this may be related to an initial immaturity of lymphoid cell production during neonatal development or an effort by the immature innate immune system to produce rapid responding cells during a time period when the adaptive immune system is not yet highly contributory. Changes in WBC counts and function have been previously reported in neonatal CF-1[15] and BALB/C mice.[21] While lymphocyte counts can rise due to increased production, mechanisms behind leukocyte tissue sequestration and destruction[18] contributing to the shifts in neutrophil/lymphocyte ratios during neonatal development are currently unclear. Another small limitation of this study is the lack of WBC differential count validation based on blood smear evaluation.

Among the RI for hematologic variables several findings were of note. For instance the initial drop in HGB before reaching adult values is in contrast to human data documenting a steady increase in utero before decreasing to adult values by 6 to 12 months of age. [22] MCV values decreased by 50% during the first 2 weeks of life in C57BL/6J mice. This is in comparison to human infants whose MCV values steadily decrease by 50% from 18 weeks of fetal gestation through one year of age. [22,23] In our study, the absolute reticulocyte counts followed a bell curve trend where newborn levels resembled adult levels early in life, followed by a 3-fold increase at p21, and then a return to low adult counts by p28. A similar

trend is seen in preterm human infants where reticulocyte counts roughly double 10 weeks after birth in preterm infants. [24] Lastly, there was an interesting trend in PLT count, first decreasing but then steadily increasing towards adult levels by p21. In contrast, in human neonates PLT counts slightly rise in utero and in general remain in a stable adult range thereafter.[22] Thus, while most murine hematology variables show similar trends to human neonates, newborn mice were thrombocytopenic during the first 2 weeks of their life. These trends are also seen in F344 rat pups[26], suggesting that platelet variation from human values may be a rodent-specific finding. However, in our study no blood smears were evaluated for platelet clumping, and thus pseudothrombocytopenia in the very small and difficult to bleed newborn mice cannot be completely ruled out.

Conclusions

In conclusion, in this study we define hematologic RI and developmental trends for newborn healthy C57BL/6J mice from birth until day 35. Hopefully, this data will allow an improved assessment of hematologic data in the investigation of complex models of human disease.

Acknowledgements

The Sysmex XT-2000iV automatic veterinary hematology analyzer used in this study was enerously provided on an on-loan basis from Sysmex America, Lincolnshire IL, USA. Support for this work was generously provided through NIH/NIDDK (DK083677 and DK097335 for SJM), NIH/NIAID (5T32AI007343 for JRW) and the Children's Miracle Network (SJM and JRW).

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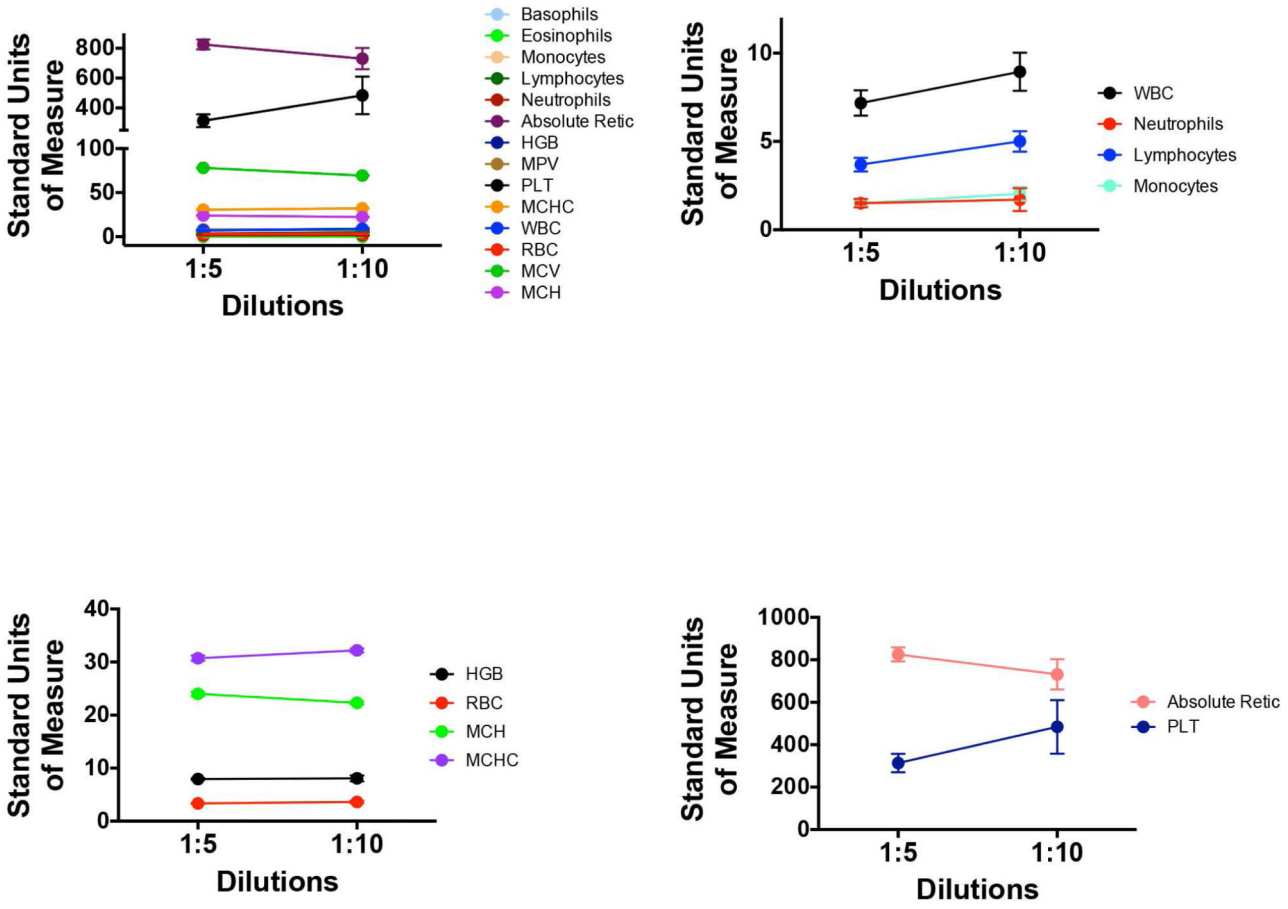

Figure 1.

Split samples of blood drawn from newborn C57Bl/6J mice on day 7 were diluted at 1:5 (n=6) or 1:10 volume (n=34) and hematologic variables were determined on the Sysmex XT-2000iV. There were no statistically significant differences between the 2 dilutions by ANOVA.

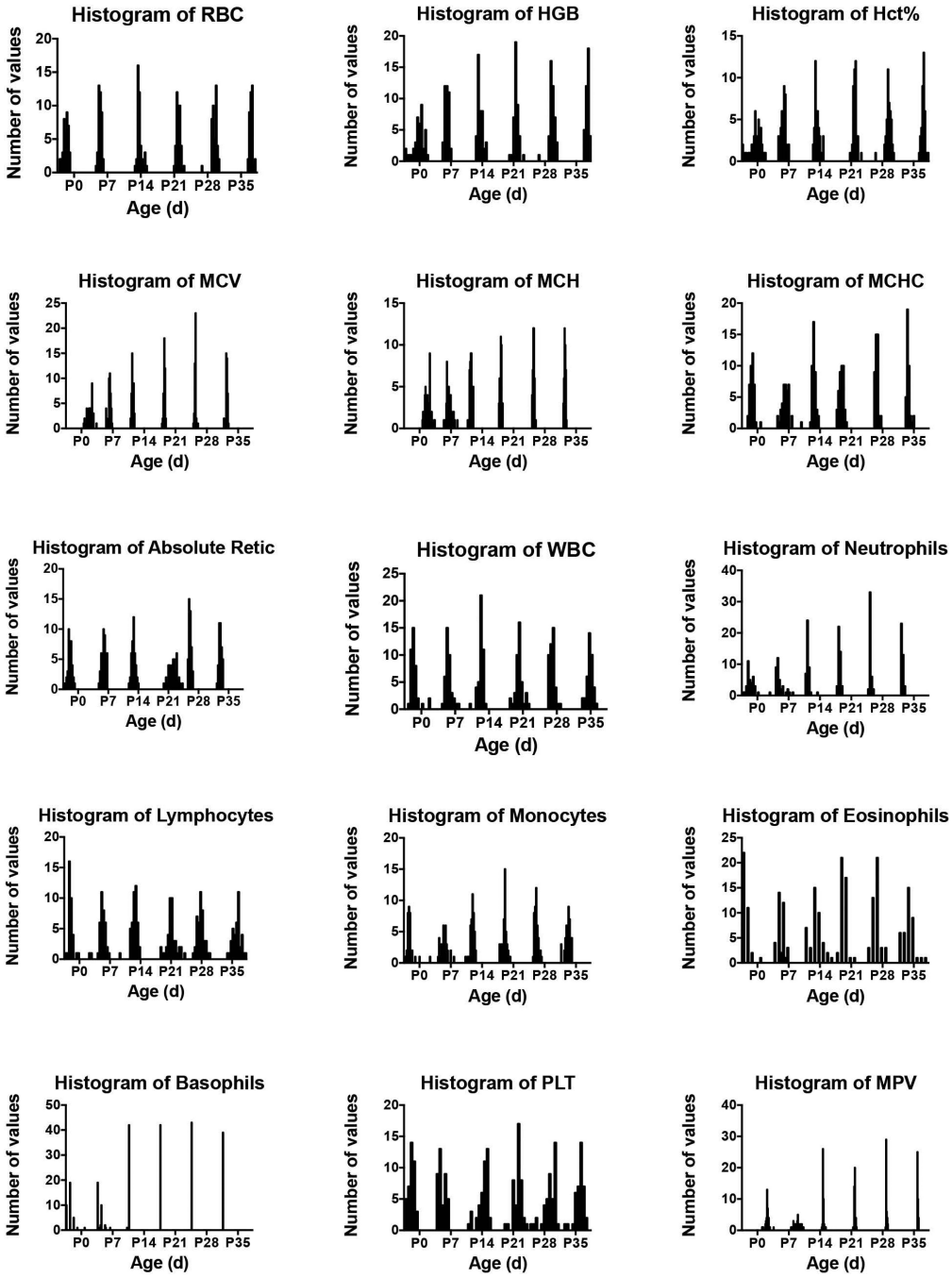

Figure 2.

Frequency distributions of hematology variables determined with the Sysmex XT-2000iV in diluted blood of newborn C57Bl/6J mice on days 0, 7, 14, 21, 28 and 35.

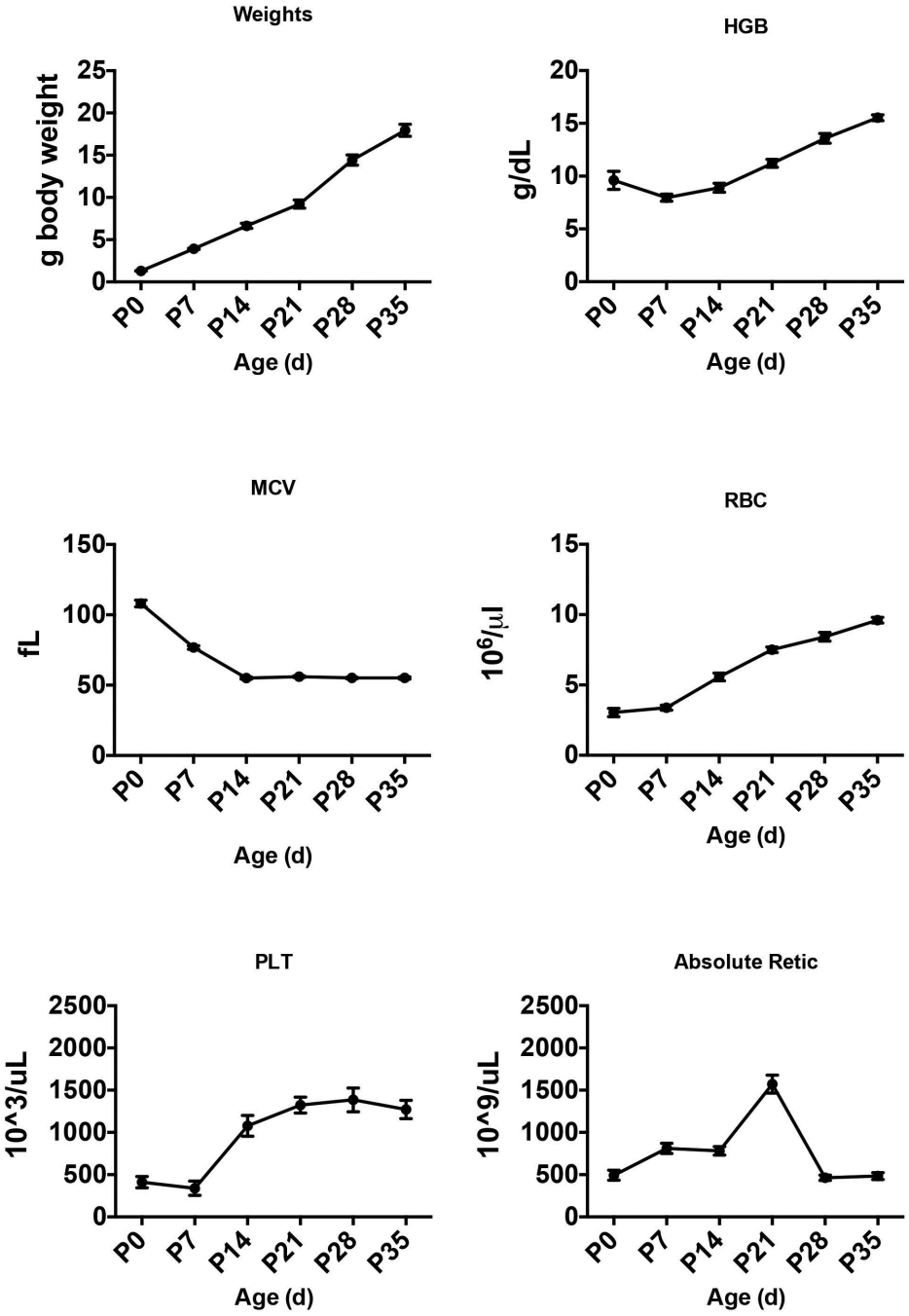

Figure 3.

Changes in means (+/−SD) in body weight and select hematology variables in newborn C57Bl/6J mice until day 35.

Retic indicates reticulocytes count.

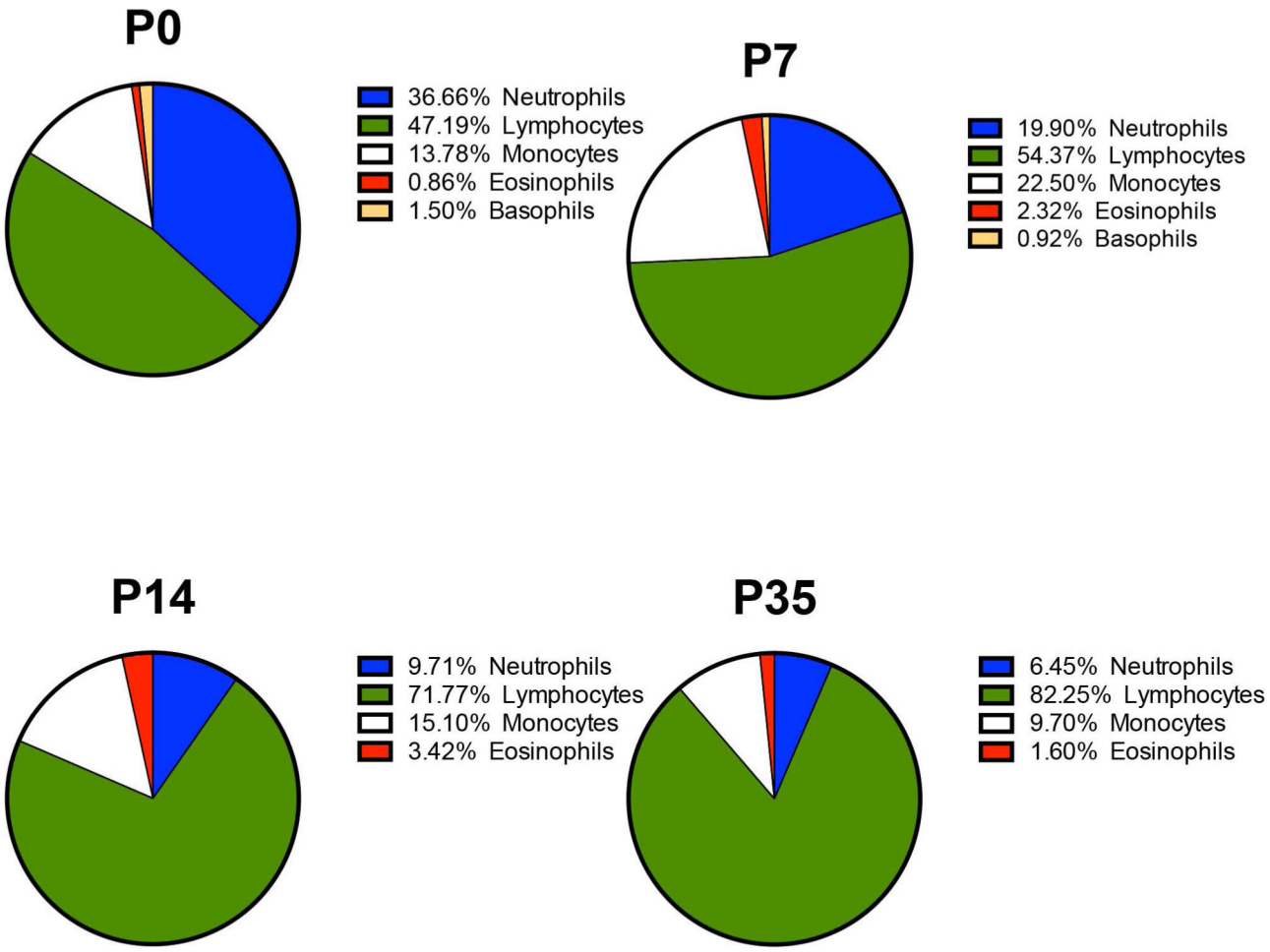

Figure 4.

Changes in differential leukocyte counts in diluted blood of newborn C57Bl/6J mice on days 0, 7, 14 and 35, as determined by the Sysmex XT-2000iV. Histograms from individual variables are listed in Figure 2.

Table 1

Sysmex XT-2000iV Performance and Capabilities

Cell Type	Variable	Reportable Range	Precision Capillary Mode	Accuracy Capillary Mode
Erythrocytes	Number of all erythrocytes	00.02-99.99 (× 10^6/μl)	4.5% or less	Within ± 8%
Reticulocytes	Reticulocyte number	0.0-999.999 (×10^3/μl)	35% or less	Within ± 25 × 10^3/μl
Hemoglobin	Hemoglobin concentration	0.1-35.0 (g/dl)	4.5% or less	Not listed
Platelets	Number of all platelets	10-9999 (× 10^3/μL)	12.0% or less	Within ± 12%
Leukocytes	Number of all leukocytes	0.1-999.99 (× 10^3/μL)	9.0% or less	Within ± 10%

Performance capabilities using capillary mode (dilution 1:5) obtained from the Sysmex Corporation. All values obtained in this study were within these reportable ranges.

Table 2

Mean, median, SD, minimum (Min) and maximum (Max) range, reference interval (RI) and confidence intervals (CI) for body weight and hematologic variables in newborn C57Bl/6J mice on Day 0 (n=40)

Analyte	Mean	Median	SD	Range (Min – Max)	95%RI	90% CI for Lower Limit	90% CI for Upper Limit
Weight (g)	1.31	1.3	0.08	1.1-1.5	NA	NA	NA
RBC (× 10^6/ μL)	3.04	3.2	0.92	0.8-4.4	0.9-4.3	0.8-1.3	4.3-4.3
HGB (g/dL)	9.6	10.00	2.70	3-14	3-13.5	3-4	13-14
HCT (%)	32.65	33.5	9.5	10-49	10-48	10-14	45-49
MCV (fL)	108.05	109.1	7.51	92.5-125	93.3-121.9	92.5-94.9	118.5-125
MCH (pg)	31.88	31.90	2.33	27.5-37.5	27.85-37.15	27.5-28.2	35.7-37.5
MCHC (g/dL)	29.54	29.55	1.54	26.5-35	26.65-33.35	26.5-27.5	31.3-35
Absolute Reticulocyte Count (× 10^3/ μL)	493.7	494.5	187.2	0-883	55-839	0-195	769-883
WBC (× 10^3/μL)	6.78	5.8	3.8	1.8-20.5	2.55-20.1	1.8-3.7	14.8-20.5
Neutrophils (× 10^3/μL)	2.30	2.10	1.38	0.0-8.2	0.0-8.2	0.0-1.0	3.6-8.2
Lymphocytes (× 10^3/μL)	3.29	2.50	2.81	0-14	0-14	0-1.5	7.3-14
Monocytes (× 10^3/μL)	0.95	0.80	0.81	0.0-4.8	0.0-4.8	0.0-0.4	2.1-4.8
Eosinophils (× 10^3/μL)	0.05	0.0	0.08	0.0-0.4	0.0-0.4	0.0-0.0	0.2-0.4
Basophils (× 10^3/μL)	0.10	0.10	0.10	0-0.5	0-0.5	0.0-0.0	0.2-0.5
Platelets (× 10^3/μL)	411.3	450.0	209.6	50-750	50-745	50-60	730-750
MPV (fL)	8.76	8.80	0.52	6.9-9.8	7.15-9.65	6.9-8.1	9.4-9.8

n indicates number of animals; MPV, mean platelet volume

Table 3

Mean, median, SD, range, reference interval (RI) and confidence interval (CI) for body weight and hematologic variables in newborn C57Bl/6J mice on Day 7 (n=40)

Analyte	Mean	Median	SD	Range (Minimum-Maximum)	95%RI	90% CI for Lower Limit	90% CI for Upper Limit
Weight (g)	3.92	3.9	0.19	3.5-4.3	NA	NA	NA
RBC ($\times 10^6$/ μL)	3.38	3.48	0.55	2.2-4.5	2.25-4.45	2.2-2.3	4.18-4.5
HGB (g/dL)	7.96	8.00	1.06	6-10	6-10	6-6.2	9.4-10
HCT (%)	25.98	27.0	4.3	17-36	17.5-35.5	17-18	31-36
MCV (fL)	76.83	77.95	3.91	68.8-83.9	68.8-83	68.8-68.9	81.8-83.9
MCH (pg)	23.76	23.60	2.21	19.4-30.4	19.95-29.5	19.4-21.4	27.6-30.4
MCHC (g/dL)	30.95	31.00	2.79	25.7-41.2	25.8-38	25.7-26.9	34.8-41.2
Absolute Reticulocyte Count ($\times 10^3$/μL)	811.5	828.5	190.4	394-1137	430-1130	394-494	1072-1137
WBC ($\times 10^3$/μL)	7.45	6.7	4.02	2-25.1	2.6-20.15	2-3.3	13.92-25.1
Neutrophils ($\times 10^3$/μL)	1.54	1.10	1.26	0.3-5.3	0.3-5.3	0.3-0.3	4.18-5.3
Lymphocytes ($\times 10^3$/μL)	3.87	3.45	2.08	0.6-13.1	0.6-13.1	0.6-1.9	6.24-13.1
Monocytes ($\times 10^3$/μL)	1.58	1.40	0.97	0.3-5.6	0.3-5.6	0.3-0.5	2.8-5.6
Eosinophils ($\times 10^3$/μL)	0.15	0.13	0.08	0.0-0.3	0.0-0.3	0.0-0.0	0.3-0.3
Basophils ($\times 10^3$/μL)	0.08	0.0	0.15	0.0-0.8	0.0-0.8	0.0-0.0	0.22-0.8
Platelets ($\times 10^3$/μL)	339.4	210.0	266.2	40-850	40-820	40-50	786-850
MPV (fL)	8.10	8.30	1.78	0.0-10.6	0.0-10.6	0.0-6.5	10-10.6

n indicates number of animals; MPV, mean platelet volume

Table 4

Mean, median, SD, range, reference interval (RI) and confidence interval (CI) for body weight and hematologic variables in newborn C57Bl/6J mice on Day 14 (n=42)

Analyte	Mean	Median	SD	Range (Minimum-Maximum)	95%RI	90% CI for Lower Limit	90% CI for Upper Limit
Weight (g)	6.64	6.6	0.69	5.3-8.1	NA	NA	NA
RBC ($\times 10^6$/ μL)	5.57	5.35	0.89	4.2-8	4.3-7.6	4.2-4.4	7.5-8
HGB (g/dL)	8.91	8.50	1.36	7-12	7-12	7-7	12-12
HCT (%)	30.62	29.0	4.64	24-42	24-41	24-25	41-42
MCV (fL)	55.05	54.70	2.56	50-60	50.9-59.3	50.0-51.0	59.1-60.0
MCH (pg)	16.04	16.00	1.02	13.7-18.2	14.3-18.0	13.7-14.8	17.9-18.2
MCHC (g/dL)	29.10	28.95	1.19	26.9-32.3	27.6-32.1	26.9-27.6	31.0-32.3
Absolute Reticulocyte Count ($\times 10^3$/μL)	781.6	775.5	160.2	434-1176	504-1075	434-522	1067-1176
WBC ($\times 10^3$/μL)	6.03	6.35	1.74	1.7-9.3	2.1-8.2	1.7-2.4	8.2-9.3
Neutrophils ($\times 10^3$/μL)	0.60	0.50	0.53	0.1-3.3	0.1-1.4	0.1-0.1	1.1-3.3
Lymphocytes ($\times 10^3$/μL)	4.28	4.40	1.28	1.5-6.6	1.6-6.5	1.5-1.8	5.9-6.6
Monocytes ($\times 10^3$/μL)	0.92	0.90	0.36	0.0-1.5	0.1-1.5	0.0-0.4	1.4-1.5
Eosinophils ($\times 10^3$/μL)	0.23	0.20	0.15	0.0-0.6	0.0-0.5	0.0-0.0	0.5-0.6
Basophils ($\times 10^3$/μL)	0.0	0.0	0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0
Platelets ($\times 10^3$/μL)	1079	1260	394.3	40-1600	110-1510	40-170	1480-1600
MPV (fL)	6.42	6.40	0.21	5.9-7.3	6.2-6.8	5.9-6.2	6.8-7.3

n indicates number of animals; MPV, mean platelet volume

Table 5

Mean, median, SD, range, reference interval (RI) and confidence interval (CI) for body weight and hematologic variables in newborn C57Bl/6J mice on Day 21 (n=42)

Analyte	Mean	Median	SD	Range (Minimum-Maximum)	95%RI	90% CI for Lower Limit	90% CI for Upper Limit
Weight (g)	9.2	9.05	1.27	6.7-11.5	NA	NA	NA
RBC ($\times 10^6$/ μL)	7.5	7.4	0.62	6-9.3	6.6-8.7	6-6.7	8.5-9.3
HGB (g/dL)	11.21	11.00	1.200	8-15	9-14	8-10	13-15
HCT (%)	42.02	42.0	3.5	33-54	36-48	33-37	47-54
MCV (fL)	56.04	56.20	2.08	50.0-59.7	51.8-59.2	50.0-52.6	58.6-59.7
MCH (pg)	14.94	14.95	0.83	13.3-16.7	13.5-16.4	13.3-13.6	16.3-16.7
MCHC (g/dL)	26.66	26.80	1.39	24.2-29.5	24.3-29.3	24.2-24.4	28.9-29.5
Absolute Reticulocyte Count ($\times 10^3$/μL)	1572	1613	339.3	776-2353	956-2150	776-1045	2148-2353
WBC ($\times 10^3$/μL)	9.71	9.4	3.33	2.3-18.4	2.4-16.6	2.3-3.3	15.7-18.4
Neutrophils ($\times 10^3$/μL)	0.70	0.70	0.31	0.1-1.4	0.2-1.4	0.1-0.2	1.4-1.4
Lymphocytes ($\times 10^3$/μL)	7.76	7.60	2.74	2.0-14.9	2.0-13.3	2.0-2.8	12.9-14.9
Monocytes ($\times 10^3$/μL)	1.10	1.20	0.45	0.1-2.3	0.1-2.0	0.1-0.2	1.7-2.3
Eosinophils ($\times 10^3$/μL)	0.15	0.10	0.07	0.0-0.4	0.0-0.3	0.0-0.1	0.2-0.4
Basophils ($\times 10^3$/μL)	0.0	0.0	0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0
Platelets ($\times 10^3$/μL)	1325	1335	301.3	310-1960	590-1770	310-930	1730-1960
MPV (fL)	6.55	6.50	0.23	6.1-7.2	6.2-7.0	6.1-6.3	6.9-7.2

n indicates number of animals; MPV, mean platelet volume

Table 6

Mean, median, SD, range, reference interval (RI) and confidence interval (CI) for body weight and hematologic variables in newborn C57Bl/6J mice on Day 28 (n=43)

Analyte	Mean	Median	SD	Range (Minimum-Maximum)	95%RI	90% CI for Lower Limit	90% CI for Upper Limit
Weight (g)	14.42	14.3	1.62	11.5-16.9	NA	NA	NA
RBC ($\times 10^6$/ μL)	8.42	8.4	1.0	3.9-10	7.3-9.8	3.9-7.4	9.7-10
HGB (g/dL)	13.58	14.00	1.48	7-16	12-16	7-12	16-16
HCT (%)	46.33	46.0	5.08	24-56	40-54	24-40	53-56
MCV (fL)	55.13	55.40	1.85	61.5-49.5	51.9-58	49.5-52.2	57.1-61.5
MCH (pg)	16.17	16.00	0.66	14.9-17.9	15.1-17.6	14.9-15.1	17.1-17.9
MCHC (g/dL)	29.34	29.40	1.01	27.5-31.7	27.7-31.7	27.5-27.7	31.1-31.7
Absolute Reticulocyte Count ($\times 10^3$/μL)	463.1	454.0	101.8	305-695	307-667	305-329	665-695
WBC ($\times 10^3$/μL)	8.93	8.8	2.18	5.2-15.5	5.5-13.4	5.2-5.7	12.6-15.5
Neutrophils ($\times 10^3$/μL)	0.61	0.60	0.26	0.2-1.4	0.2-1.3	0.2-0.3	1.2-1.4
Lymphocytes ($\times 10^3$/μL)	6.99	6.90	1.89	3.3-12.2	3.6-10.6	3.3-4.1	10.4-12.2
Monocytes ($\times 10^3$/μL)	1.15	1.10	0.36	0.6-2.2	0.7-2.1	0.6-0.7	1.9-2.2
Eosinophils ($\times 10^3$/μL)	0.18	0.20	0.09	0.0-0.4	0.0-0.4	0.0-0.0	0.4-0.4
Basophils ($\times 10^3$/μL)	0.0	0.0	0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0
Platelets ($\times 10^3$/μL)	1386	1460	456.6	90-1960	230-1890	90-340	1890-1960
MPV (fL)	6.69	6.70	0.16	6.4-7.1	6.5-7.1	6.4-6.5	7.0-7.1

n indicates number of animals; MPV, mean platelet volume

Table 7

Mean, median, SD, range, reference interval (RI) and confidence interval (CI) for body weight and hematologic variables in newborn C57Bl/6J mice on Day 35 (n=39)

Analyte	Mean	Median	SD	Range (Minimum-Maximum)	95%RI	90% CI for Lower Limit	90% CI for Upper Limit
Weight (g)	17.95	17.9	1.73	14.4-20.6	NA	NA	NA
RBC ($\times 10^6$/ μL)	9.61	9.7	0.61	8.5-11.2	8.5-11.2	8.5-8.8	10.5-11.2
HGB (g/dL)	15.15	16.00	2.60	14-17	14-17	14-14	17-17
HCT (%)	52.9	54.0	3.17	45-61	45-61	45-48	58-61
MCV (fL)	55.13	55.2	2.51	47.1-60.2	47.1-60.2	47.1-53.1	58.3-60.2
MCH (pg)	16.19	16.2	0.57	15.2-17.5	15.2-17.5	15.2-15.2	17.0-17.5
MCHC (g/dL)	29.4	29.2	1.22	27.8-33.3	27.8-33.3	27.8-28.0	31.5-33.3
Absolute Reticulocyte Count ($\times 10^3$/μL)	483.4	478.0	122.6	223-727	223-727	223-285	665-727
WBC ($\times 10^3$/μL)	12.14	12.4	2.55	6.7-17.1	6.7-17.1	6.7-8.1	16.1-17.1
Neutrophils ($\times 10^3$/μL)	0.76	0.70	0.29	0.3-1.5	0.3-1.5	0.3-0.4	1.3-1.5
Lymphocytes ($\times 10^3$/μL)	10.00	10.30	2.23	5-14.7	5-14.7	5.0-6.5	13.3-14.7
Monocytes ($\times 10^3$/μL)	1.18	1.30	0.51	0.0-2.1	0.0-2.1	0.0-0.0	1.9-2.1
Eosinophils ($\times 10^3$/μL)	0.20	0.20	0.13	0.0-0.6	0.0-0.6	0.0-0.0	0.4-0.6
Basophils ($\times 10^3$/μL)	0.0	0.0	0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0
Platelets ($\times 10^3$/μL)	1241	1345	380.5	180-1800	180-1800	180-790	1620-1800
MPV (fL)	6.70	6.70	0.11	6.5-6.9	6.5-6.9	6.5-6.6	6.9-6.9

n indicates number of animals; MPV, mean platelet volume