**Title:** Estimation of Gestational Age-Specific Reference Intervals for Coagulation Assays in a Neonatal Intensive Care Unit Using Real-World Data

**Running Header:** Coagulation Reference Interval Estimation in a NICU

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***Abstract***

**Background**

Interpretation of coagulation testing in neonates currently relies on reference intervals (RIs) defined from older patient cohorts. Direct RI studies are difficult, but indirect estimation may allow us to infer normative neonatal distributions from routinely collected clinical data.

**Methods**

We analyzed first-in-life coagulation testing results from all patients admitted to a level IV neonatal intensive care unit between 1/1/2018-1/1/2024. Results obtained after transfusion of any blood product were excluded. Indirect RIs were estimated across gestational age groups using refineR, and compared to currently reported intervals for patients less than one year of age.

**Results**

Prothrombin times (PT) and international normalized ratios (INR) were available for 1,128 neonates, while activated partial thromboplastin times (aPTT) were available for 790 neonates. The indirect RI was 10-25s in preterm, 10-22s in term, and 10-24s in all neonates for PT, 0.7-2.1 in preterm, 0.8-1.8 in term, and 0.8-1.9 in all neonates for INR, and 25-68s in preterm, 25-58s in term, and 25-62s in all neonates for aPTT. Compared to our current intervals, the indirect RIs would flag 58% fewer PT, 43% fewer INR, and 17% fewer aPTT results as abnormal.

**Conclusions**

Indirectly estimated RIs in neonates admitted to intensive care show substantial divergence from current, first-year-of-life RIs, leading to an abundance of abnormal flags. The associations between these flags and provider behavior, transfusion practice, or clinical outcomes is an area of future exploration.

***Introduction***

Disorders of hemostasis contribute to significant morbidity and mortality in neonates, especially those born prematurely(1,2). Laboratory results, such as the prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (aPTT), often play a crucial role in screening for abnormalities in hemostasis and guiding transfusion of blood products. However, their clinical interpretation is fraught with pitfalls, including insensitivity to the role of platelets, endothelium, and other factors in achieving hemostasis, the methodological variation across time and institutions, and perhaps most importantly, the difficulties associated with establishing a “normal” range, or reference interval (RI).

RIs represent the range of values occupied by a healthy population. They are classically defined by the middle 95% distribution of values measured in adult volunteers. These “direct” RIs can lack generalizability when applied to new populations but defining appropriate pediatric- and/or neonate-specific RIs is a Herculean task(3–5)(3–5). Despite this, age-related changes in physiology often necessitate that multiple age-specific reference intervals be established. The youngest age bracket in the current reference intervals for coagulation testing at our institution span the entire first year of life. However, differences in hemostatic balance in the neonatal period represent a pitfall in this approach, particularly in first weeks of life(6–10).

Challenges inherent to direct RI estimation have motivated development of computationally sophisticated “indirect” methods, which estimate the distribution of non-pathologic results generated through routine clinical care. One such approach is *refineR*, which has shown to outperform older indirect and direct approaches, but has not yet been applied to coagulation testing data(11,12). The *refineR* approach uses all real-world data as inputs and leverages a sophisticated curve smoothing approach to separate the pathologic results from non-pathologic. An indirect reference interval can then be estimated on that non-pathologic fraction, without requiring upfront filtering of healthy patients. We hypothesized that the current RIs for PT/INR and aPTT, which span the full first year of life, were leading to a high proportion of results in neonatal populations being flagged as abnormal. If true, this could obfuscate bleeding risk assessment, motivate unnecessary transfusions, and contribute to “alert fatigue” in clinical providers. Given the risks of transfusion(1,2,13,14) and the unclear association between laboratory values and bleeding outcomes(15–18), a more robust analysis is sorely needed.

We aimed to assess the concordance between the normative distribution of neonatal PT, INR, and aPTT results estimated by *refineR* with those currently used at our institution and compare them to previously published direct estimates in similar populations(15,17,19–21). This work represents a crucial first step in the critical reappraisal of the clinical decision-making framework surrounding hemostasis evaluation in neonates at our institution, and represent a convenient and efficient method by which context-specific reference intervals could be defined more broadly. We hope that by making the anonymized data and code publicly available, we can encourage replication and reproduction at other institutions and facilitate the development of more evidence-based reference intervals and transfusion practices.

***Methods***

*Data Collection and Processing*

This study was reviewed and approved by the Washington University Institutional Review Board (IRB ID: 202402053). Data from routine clinical care of all neonates admitted to the neonatal intensive care unit for whom a PT, INR, or aPTT was performed between 1/1/2018 and 1/1/2024 (n = 9,467 results, 1,189 unique patients) were extracted from the electronic medical record. To reduce the risk of ascertainment bias, only the first measurement of each assay was included. Results performed after the administration of a blood product were removed (n = 72 results). Non-numerical results (e.g. “See Comment”, “>250s”) were excluded. Gestational age (GA) was binned into preterm and term using 37 weeks as a cut-off.

All results were performed on fresh plasma samples collected directly from neonates, as umbilical cord blood coagulation testing is not performed at our institution. Results prior to July 2021 were analyzed on the viscosity-based STA Compact Max (Diagnostica Stago. Parsippany, NJ, USA). More recent results used a nephelometry-based ACL Top (Instrumentation Laboratory. Bedford, MA, USA). The difference in medians for PT and aPTT were both less than the total allowable error of 15%[(20)](https://www.zotero.org/google-docs/?pxNBae), so data from before and after the instrumentation change were combined to increase sample size. Indirect reference interval estimation was performed using *refineR(11)*, with the modified Box-Cox method(22). These indirect RIs were then compared to our currently reported intervals for patients <1 year old, and several previously published direct RIs(15,17,19–21).

All analysis was performed using *R 4.3.0(23)* within the *tidyverse(24)* framework. Color palettes were chosen from Crameri’s Scientific Colourmaps(25). Code is available at https://github.com/nspies13/nicu\_coag\_reference\_intervals, while anonymized input data is available on FigShare at 10.6084/m9.figshare.25484761(26).

***Results and Discussion***

*Demographic Summary*

There were 1,117 neonates with numeric PT/INR data reported prior to receiving a transfusion, and 790 with aPTTs. The median GA was 36 weeks (IQR: 29-38w). 55% were delivered preterm. The median birth weights were 2,532g for neonates with measured PT/INR, and 2,637g for those with aPTT.

*The Distribution of First Coagulation Results Varies Across Gestational Age Groups*

Only first-in-life results were included for analysis.88% of results were collected within the first 48 hours of life, 98% within the first 7 days, and 100% within the first 14 days of life. **Figure 1** demonstrates the results of each assay by GA. Pearson correlations were -0.28, -0.27, and -0.37 for PT, INR, and aPTT, respectively. From 24w to 41w, while the lower percentiles show modest decreases, the 97.5th percentiles markedly decrease from 29s to 22s for PT, from 2.5 to 1.8 for INR, and from 105s to 58s for aPTT.

*Inferring the Distributions of Non-Pathological Results*

**Figure 2** displays the *refineR-*estimated pathological and non-pathological distributions for each assay in preterm and term neonates. RI estimates were made using only the non-pathological fraction. The RI for PT was [9.5 - 24.6s] for preterm, [10 - 22s] for term, and [10 - 23.6s] for all neonates. The RI for INR was [0.6 - 1.7] for preterm, and [0.8 - 1.9] for term and all neonates. The RI for aPTT was [23.7 - 67.8s] for preterm, [24.5 - 58.6s] for term, and [25.7 - 58.7s] for all neonates.

**Figure 3** compares the indirect RI estimates with currently reported or previously published RIs. The lower limits of each interval show minor variation. However, marked divergence is observed in the upper limit for each assay across intervals. For PT, the estimated upper limit was 25s for preterm, 22s for term, and 24s for all neonates. For INR, the estimated upper limits were 2.1, 1.8, and 1.9 for preterm, term, and all neonates. For aPTT, the upper limit of the indirect RIs were 68s for preterm, 58s for term, and 62s for all neonates.

*Discrepancies Between Intervals Affect a Substantial Proportion of Results*

**Figure 4** compares the proportion of results that would be flagged as abnormal by the current, 0-1 year reference interval compared to the indirectly estimated neonatal alternative. Six (0.16%) results below the current RI were counted as “normal” for simplicity.

For PT, the current RI flags 73% of results as abnormal for both preterm and term neonates. The gestational age-specific indirect RIs (GA IRIs) would flag 17% for preterm, and 13% for term neonates. For INR (not adjusted by a neonate-specific mean PT), the current RI flags 61% of results for preterm and 54% for term neonates. In contrast, the GA IRIs would flag 16% for preterm and 12% for term neonates. For aPTT, the current RI flags 31% of results for preterm and 22% for term neonates, while the GA IRIs would flag 11% for preterm and 9% for term neonates.

Altogether, applying the GA IRIs resulted in 58% fewer PT, 43% fewer INR, and 17% fewer aPTT results being flagged as abnormal as compared to our current, <1 year intervals. This marked difference in the current and indirectly estimated reference intervals is best contextualized by considering that a typical direct interval is designed to flag 5% of results as abnormal.

These abnormal flags imply the need for an action to be taken, which may range from closer monitoring through serial blood draws to transfusion of one or more blood products. However, an overabundance of abnormally flagged results may contribute to “alert fatigue” and subsequent delayed or absent response to abnormal results. Wider RIs may help improve clinical outcomes through a reduction in alert fatigue, but further analysis is required to explore this hypothesis. We believe this work is a much-needed first step in optimizing the utility of these assays – recognizing the limited scope that they can provide towards comprehensive evaluation of hemostasis.

Strengths of this work include its novelty as the first (to our knowledge) indirect reference interval study for these hemostasis assays in neonates, the order-of-magnitude larger scale relative to prior direct RI studies[(8–13)](https://www.zotero.org/google-docs/?aPiOyH), and the relative ease in reproducing such an analysis at one’s own institution using the open-source *refineR* package(11,12) and our provided code.

That said, this work is not without its limitations. First, the PT, INR, and aPTT provide only a narrow window into the full hemostatic balance of the neonate, where hypoactive platelets and lower concentrations of coagulation factors are balanced out by decreases in proteins C, S, and antithrombin, as well as increases in hematocrit and von Willebrand factor(7,8). We hypothesize that, because the PT/INR and aPTT are routinely used as screening tests, and the currently reported intervals are too narrow to adequately capture normative neonatal variation, a portion of these results are being erroneously flagged as abnormal in patients without an increased bleeding risk. By extension, these abnormal flags in coagulation assays may be misinterpreted by providers as signaling a disturbance in hemostasis where none exists, and may even motivate unnecessary repeat phlebotomy, confirmatory testing, or plasma transfusion(3,5,17).

Altogether, the clinical decision-making surrounding bleeding and transfusion, and the role that these and other laboratory tests, reference ranges, and abnormal flags play in those decisions, should be explored more thoroughly. Additionally, while indirect RIs aim to extract pathologic from non-pathologic results, the extent to which these non-pathologic distributions represent neonates who are not at increased risk of bleeding requires a more robust outcomes-based study. This outcomes-based analysis is a critical next step in better evaluating these indirect RIs, and should come before any clinical implementation is pursued. Investigating the incidence of both short- and long-term morbidity, their association with transfusion practices, and the role for this approach across a wider range of assays presents ample opportunity to refine and optimize the approach prior to a prospective validation.

Altogether, we observed that the normative distributions of PT, INR, and aPTT in in neonates differ substantially from the currently reported reference intervals, which target all patients under one year of age. This leads to an overwhelming proportion of results being flagged as abnormal, motivating an exploration of neonate-specific reference intervals. The refineR-based approach produced estimated intervals in line with previously reported direct approaches, with substantially less investment. While future efforts will be needed to assess whether these indirect RIs would reduce unnecessary transfusion or improve care, we believe this work is a practical first step in evaluating adoption of population-specific reference intervals, and motivates the continued exploration of this important problem.

***Declaration of Competing Interests***

The authors have no competing interests to disclose.

***Author Contributions***

N.L., Z.V., and N.C.S contributed to concept and study design, figure generation, and drafting of the paper. C.M and N.C.S contributed to data generation, interpretation, and analysis. C.E, S.R, and D.J.D. contributed to critical interpretations of study design, methods, and results, as well as intellectual contributions to the discussion. All authors contributed to the preparation of the final manuscript and figures.

***Ethics Statement***

This work was reviewed and approved by the Washington University Institutional Review Board (IRB ID: 202402053).

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***Figure Legends***

**Figure 1: Observed Measurements for First-in-Life Coagulation Assay Results Across Gestational Ages.** 1,117 PT/INR results and 790 aPTT results are shown as a function of gestational age. The smoothed middle 95th percentile (dark red, outside curves), interquartile range (gold, middle curves), and median (tan, inner curve) is overlaid atop the scatterplots. Pearson correlations (r) are shown in the top left corner of each plot.

**Figure 2: Inference of Pathologic and Non-Pathologic Distributions and Indirect Reference Intervals with refineR.** Distributions of non-pathologic results (blue) were estimated for each test and gestational age class using refineR. Pathologic distributions (red) were calculated by subtracting total counts from the proportion inferred to be non-pathologic. Resulting indirect reference intervals are represented by dashed vertical lines.

**Figure 3: Current and Indirectly Estimated Reference Intervals.** Comparison between currently reported (black boxes), indirectly estimated (red boxes), and previously published direct reference intervals (gray boxes) for each assay and population.

**Figure 4: Proportion of Results Flagged as Abnormal by Each Reference Interval Definition.** The proportion of results flagged as normal (gray) and abnormal (red) for the currently reported reference intervals (Current) and those indirectly estimated by *refineR* (Indirect).