**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*** (241/250)

**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.

**Results**

Prothrombin times (PT) and international normalized ratios (INR) were available for 1,117 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 show substantial divergence from current RIs in their upper limits, leading to substantial reductions in 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)](https://www.zotero.org/google-docs/?YvOBq4). 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, and are classically defined by the middle 95% distribution of values measured in healthy 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)](https://www.zotero.org/google-docs/?h7eT56). Current RIs for coagulation testing often rely on direct estimates from adult populations, despite evidence of developmental differences in hemostasis[(6,7)](https://www.zotero.org/google-docs/?GbVpKu) and prior direct studies in various neonatal populations[(8–13)](https://www.zotero.org/google-docs/?4L2AWl). Challenges inherent to direct RI estimation have motivated development of computationally sophisticated “indirect” methods[(14–16)](https://www.zotero.org/google-docs/?AufwMy), 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[(16)](https://www.zotero.org/google-docs/?WP30R0). However, these indirectly estimated reference intervals are vulnerable to ascertainment bias, especially when the patient populations being tested are largely not healthy, such as extremely premature neonates.

We hypothesized that the current RIs for PT/INR and aPTT 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[(1,2,17,18)](https://www.zotero.org/google-docs/?qCjs9H) and the unclear association between laboratory values and bleeding outcomes[(12,13,19)](https://www.zotero.org/google-docs/?Ljj8jO), 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. 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. 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 transfusion guidelines.

***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.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*[(16)](https://www.zotero.org/google-docs/?f6gx8W), with the modified Box-Cox method.

Using *refineR*, the distribution of non-pathologic results for PT, INR, and aPTT was approximated, and middle 95th percentile estimated. These indirect RIs were then compared to the currently reported RIs and the proportion of results flagged as abnormal was calculated for each interval.

Finally, to assess the degree to which a simple change in population mean PT affected the indirect RI for INR, a comparison between indirect RIs for INR calculated using the current population mean PT (11.8s) was compared to those calculated using the geometric mean PT of all neonates (17.5s).

All analysis was performed using *R 4.3.0*[(21)](https://www.zotero.org/google-docs/?6juDxu) within the *tidyverse*[(22)](https://www.zotero.org/google-docs/?BrY1xn) framework. Color palettes were chosen from Crameri’s Scientific Colourmaps[(24)](https://www.zotero.org/google-docs/?ODcoZo). Code is available at XXX, while an anonymized input data is available at XXX.

***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 and interquartile range 29 to 38 weeks for both assays. 55% of patients were delivered preterm, while 45% were delivered at term. The median birth weight was 2,532g for neonates with measured PT/INR, and 2,637g for aPTT values. No significant differences across age or weight were observed between the subset of patients with and without an aPTT result.

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

**Figure 1** demonstrates the scatterplots of all first-in-life coagulation test results by GA. Each assay’s result distribution shows gradual decreases as gestational age increases, with Pearson correlations of -0.28, -0.27, and -0.37 for PT, INR, and aPTT, respectively. From 24w to 41w, while the 2.5th and 25th percentiles show little change and the medians 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. A separate interval was calculated for all neonates to reflect the current practice, but not shown.

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** overlays the indirect RI estimates with currently reported RIs. The lower limits of the estimated intervals show relatively little divergence from the current intervals. However, marked divergence is observed in the upper limit for each assay across all age groups.

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. When an adjustment was added using the geometric mean of the PT for all neonates, as opposed to all patients (17.5s vs. 11.8s), for INR calculation, the upper limits of the indirect RIs were 1.8, 1.6, and 1.7 for preterm, term, and all neonates, respectively. 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 standard compared to the indirectly estimated 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 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 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)](https://www.zotero.org/google-docs/?37cikD). Given that these other factors are not routinely assessed, the reduction in abnormal flags for PT/INR may reduce the frequency at which they are mis-interpreted as imbalanced hemostasis and increased bleeding risk[(12,13)](https://www.zotero.org/google-docs/?LdUNe4). However, the clinical decision-making surrounding bleeding and transfusion, and the role that these laboratory results, 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. 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 older patients differ substantially from those in neonates across all gestational ages, leading to a potentially overwhelming proportion of results being flagged as abnormal. While future efforts will be needed to assess whether these indirect RIs would reduce unnecessary transfusion or improve care, this work objectively quantifies the discordance between current and indirect intervals and motivates the need for the continued exploration of this important problem.

***Funding and Disclosures:***

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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 reference intervals (gray boxes) and indirectly estimated RIs (black lines) for each assay and population. Indirect RIs for INR calculated after adjusting the PT ratios using a neonate-specific geometric mean are shown below the unadjusted intervals in gray.

**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).