

## Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

**eTable 1. Definitions**

| Event                                 | Definitions   |
|---------------------------------------|---|
| Major congenital anomalies            | Chromosomal anomalies, syndromes likely affecting long-term outcome, major malformations requiring surgical correction during newborn period, or cyanotic heart defects   |
| Bleeding disorder                     | Any genetic or congenital disorders associated with a higher risk of bleeding   |
| Major bleeding                        | <p>Any of the following bleedings:</p> <ul style="list-style-type: none"> <li>– Intraventricular haemorrhage (IVH) is defined as IVH Grade 3 (extension of bleeding involving &gt;50% of ventricular area or dilation of ventricle) or IVH Grade 4/IPE (extension of bleeding into surrounding parenchyma) <sup>1</sup></li> <li>– Intracranial haemorrhage (non-IVH) is defined as a major bleeding if any of the following apply: neurosurgical intervention is required; radiological imaging showing a midline shift; clinical signs and symptoms of an oxygen deficit with significant derangement of laboratory investigations</li> <li>– Pulmonary bleeding is defined as acute fresh blood through the endotracheal tube associated with increased ventilatory requirements or the need for intubation and ventilation</li> <li>– Frank rectal bleeding is defined as macroscopic faecal bleed (not if only occult positive)</li> </ul> |
| Sepsis                                | Culture proven sepsis   |
| NEC                                   | At least NEC Stage III according to the modified Bell Staging Criteria <sup>2</sup>   |
| High flow respiratory support         | High flow (>2L/min) FiO <sub>2</sub> ≤0.3 or FiO <sub>2</sub> >0.3 by non-invasive respiratory support, including continuous positive airway pressure, biphasic intermittent positive airway pressure (synchronized or unsynchronized) and nasal high flow  |
| Invasive mechanical ventilation       | By invasive respiratory support for which the infant is intubated, including conventional mechanical ventilation and high frequency oscillation (HFO)   |
| Transfusion associated adverse effect | Any adverse effects that the local investigator deemed potentially associated with the preceeding transfusion.  |

**eTable 2.** RBC Transfusion Thresholds (Hb in g/L) Tested in the ETTNO and TOP Trials <sup>3,4</sup>

| Respiratory Support                                     | ETTNO (Franz et al. JAMA 2020) |  | TOP (Kirpalani et al. NEJM 2020) |  |
|---|--------------------------------|--|----------------------------------|--|
|   | Critical                       | RBC Thresholds   | Respiratory Support              | RBC Thresholds   |
| Room air  | No                             | Day 1-7<br><i>Liberal: 117</i><br><i>Restrictive: 93</i>   | No                               | Day 1-7<br><i>Liberal: 120</i><br><i>Restrictive: 100</i>  |
|   |                                | Day 8-21<br><i>Liberal: 103</i><br><i>Restrictive: 80</i>  |                                  | Day 8-14<br><i>Liberal: 110</i><br><i>Restrictive: 85</i>  |
|   |                                | >Day 21<br><i>Liberal: 93</i><br><i>Restrictive: 70</i>    |                                  | >Day 15<br><i>Liberal: 100</i><br><i>Restrictive: 70</i>   |
| Low flow ( $\leq 2\text{L/min}$ ) $\text{FiO}_2 > 0.21$ | No                             | Day 1-7<br><i>Liberal: 117</i><br><i>Restrictive: 93</i>   | Yes/No*                          | Day 1-7<br><i>Liberal: 130</i><br><i>Restrictive: 100</i>  |
|   |                                | Day 8-21<br><i>Liberal: 103</i><br><i>Restrictive: 80</i>  |                                  | Day 8-14<br><i>Liberal: 125</i><br><i>Restrictive: 85</i>  |
|   |                                | >Day 21<br><i>Liberal: 93</i><br><i>Restrictive: 70</i>    |                                  | >Day 15<br><i>Liberal: 110</i><br><i>Restrictive: 70</i>   |
| High flow ( $> 2\text{L/min}$ ) $\text{FiO}_2 \leq 0.3$ | Yes/No**                       | Day 1-7<br><i>Liberal: 137</i><br><i>Restrictive: 93</i>   | Yes                              | Day 1-7<br><i>Liberal: 130</i><br><i>Restrictive: 110</i>  |
|   |                                | Day 8-21<br><i>Liberal: 123</i><br><i>Restrictive: 80</i>  |                                  | Day 8-14<br><i>Liberal: 125</i><br><i>Restrictive: 100</i> |
|   |                                | >Day 21<br><i>Liberal: 113</i><br><i>Restrictive: 70</i>   |                                  | >Day 15<br><i>Liberal: 110</i><br><i>Restrictive: 85</i>   |
| High flow ( $> 2\text{L/min}$ ) $\text{FiO}_2 > 0.3$    | Yes***                         | Day 1-7<br><i>Liberal: 137</i><br><i>Restrictive: 113</i>  | Yes                              | Day 1-7<br><i>Liberal: 130</i><br><i>Restrictive: 110</i>  |
|   |                                | Day 8-21<br><i>Liberal: 123</i><br><i>Restrictive: 100</i> |                                  | Day 8-14<br><i>Liberal: 125</i><br><i>Restrictive: 100</i> |
|   |                                | >Day 21<br><i>Liberal: 113</i><br><i>Restrictive: 90</i>   |                                  | >Day 15<br><i>Liberal: 110</i><br><i>Restrictive: 85</i>   |
| Invasive mechanical ventilation                         | Yes                            | Day 1-7<br><i>Liberal: 137</i><br><i>Restrictive: 113</i>  | Yes                              | Day 1-7<br><i>Liberal: 130</i><br><i>Restrictive: 110</i>  |
|   |                                | Day 8-21<br><i>Liberal: 123</i><br><i>Restrictive: 100</i> |                                  | Day 8-14<br><i>Liberal: 125</i><br><i>Restrictive: 100</i> |
|   |                                | >Day 21<br><i>Liberal: 113</i><br><i>Restrictive: 90</i>   |                                  | >Day 15<br><i>Liberal: 110</i><br><i>Restrictive: 85</i>   |

High flow ( $> 2\text{L/min}$ )  $\text{FiO}_2 \leq 0.3$  or  $\text{FiO}_2 > 0.3$  by non-invasive respiratory support, including continuous positive airway pressure (CPAP), biphasic intermittent positive airway pressure (synchronized or unsynchronized) and nasal high flow. Mechanical

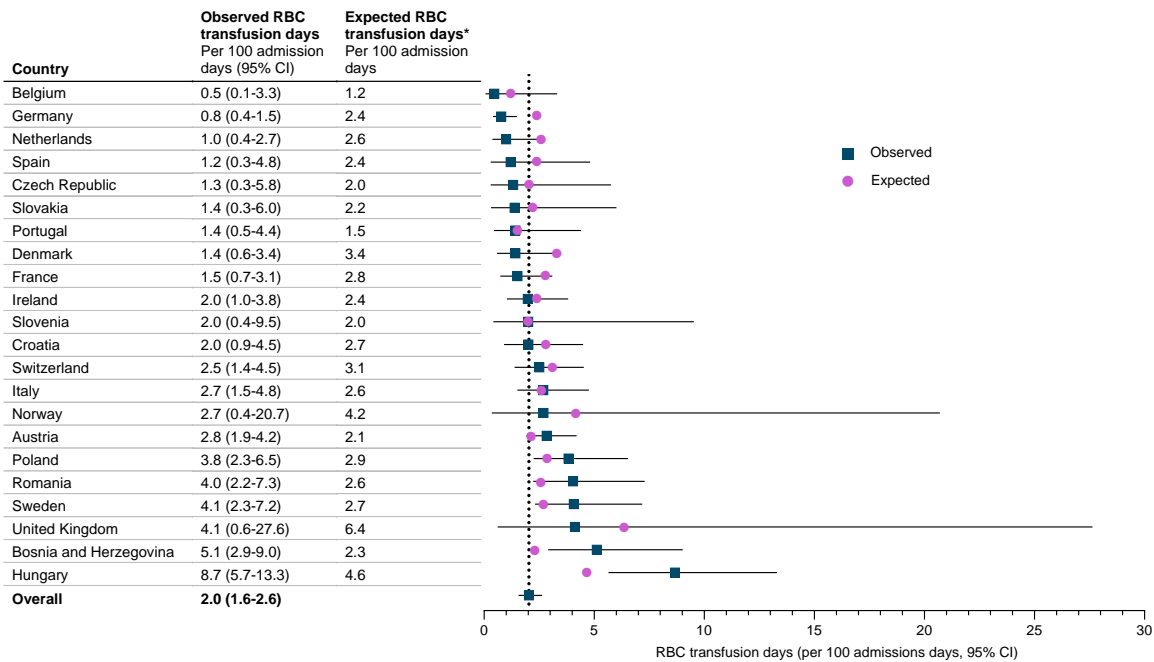
ventilation by invasive respiratory support for which the infant is intubated, including conventional mechanical ventilation and high frequency oscillation. \*Categorized as both 'respiratory support' and 'no respiratory support' per TOP definition, respiratory support if flow  $\geq 1$  liter per min. \*\*Categorized as both 'critical' and 'noncritical' per ETTNO definition, critical if CPAP with  $\text{FiO}_2 > 0.25$  for  $> 12$  hours per 24 hours. Highest liberal and lowest restrictive transfusion threshold values were selected if scenarios could be categorized as both. \*\*\*Categorized as 'critical' based on expert opinion, considering that the majority of infants on high flow ( $> 2\text{L/min}$ )  $\text{FiO}_2 > 0.3$  are critically ill and require respiratory support for more than 12 hours per 24 hours. ETTNO thresholds were converted using the formula:  $\text{Hb (g/L)} = \text{Hematocrit (Hct) (\%)} / 0.3$ .

**eTable 3.** Participating Center Characteristics

| Country                | Participating centers<br>(n (% of total)) | Centers with<br>academic status<br>(n (%)) | Centers that perform<br>NEC surgery<br>(n (%)) | Large centers*<br>(n (%)) |
|------------------------|---|--|--|---------------------------|
| Austria                | 2 (3%)                                    | 2/2 (100)                                  | 2/2 (100)                                      | 1/2 (50)                  |
| Belgium                | 1 (2%)                                    | 1/1 (100)                                  | 1/1 (100)                                      | 1/1 (100)                 |
| Bosnia and Herzegovina | 1 (2%)                                    | 1/1 (100)                                  | 1/1 (100)                                      | 1/1 (100)                 |
| Croatia                | 2 (3%)                                    | 2/2 (100)                                  | 1/2 (50)                                       | 1/2 (50)                  |
| Czech Republic         | 2 (3%)                                    | 2/2 (100)                                  | 1/2 (50)                                       | 2/2 (100)                 |
| Denmark                | 1 (2%)                                    | 1/1 (100)                                  | 1/1 (100)                                      | 1/1 (100)                 |
| France                 | 6 (9%)                                    | 3/6 (50)                                   | 4/6 (67)                                       | 3/6 (50)                  |
| Germany                | 4 (6%)                                    | 4/4 (100)                                  | 3/4 (75)                                       | 2/4 (50)                  |
| Hungary                | 3 (5%)                                    | 3/3 (100)                                  | 1/3 (33)                                       | 3/3 (100)                 |
| Ireland                | 2 (3%)                                    | 2/2 (100)                                  | 0/2 (0)  | 2/2 (100)                 |
| Italy                  | 8 (13%)                                   | 8/8 (100)                                  | 7/8 (88)                                       | 1/8 (13)                  |
| Netherlands            | 3 (5%)                                    | 3/3 (100)                                  | 2/3 (67)                                       | 3/3 (100)                 |
| Norway                 | 2 (3%)                                    | 2/2 (100)                                  | 1/2 (50)                                       | 0/2 (0)                   |
| Poland                 | 5 (8%)                                    | 4/5 (80)                                   | 3/5 (60)                                       | 1/5 (20)                  |
| Portugal               | 2 (2%)                                    | 2/2 (100)                                  | 2/2 (100)                                      | 0/2 (0)                   |
| Romania                | 3 (5%)                                    | 2/3 (67)                                   | 0/3 (0)  | 3/3 (100)                 |
| Slovakia               | 2 (3%)                                    | 2/2 (100)                                  | 1/2 (50)                                       | 0/2 (0)                   |
| Slovenia               | 2 (3%)                                    | 2/2 (100)                                  | 1/2 (50)                                       | 1/2 (50)                  |
| Spain                  | 6 (9%)                                    | 6/6 (100)                                  | 5/6 (83)                                       | 3/6 (50)                  |
| Sweden                 | 3 (5%)                                    | 3/3 (100)                                  | 2/3 (67)                                       | 2/3 (67)                  |
| Switzerland            | 2 (3%)                                    | 2/2 (100)                                  | 2/2 (100)                                      | 1/2 (50)                  |
| United Kingdom         | 2 (3%)                                    | 2/2 (100)                                  | 1/2 (50)                                       | 1/2 (50)                  |
| Overall                | 64/64 (100%)                              | 59/64 (92%)                                | 42/64 (66%)                                    | 33/64 (52%)               |

\*Large if center cares for more than 100 preterm infants born below 32 weeks gestation annually.

**eFigure 1.** RBC Transfusion Day Prevalence Rates After the First 28 Postnatal Days



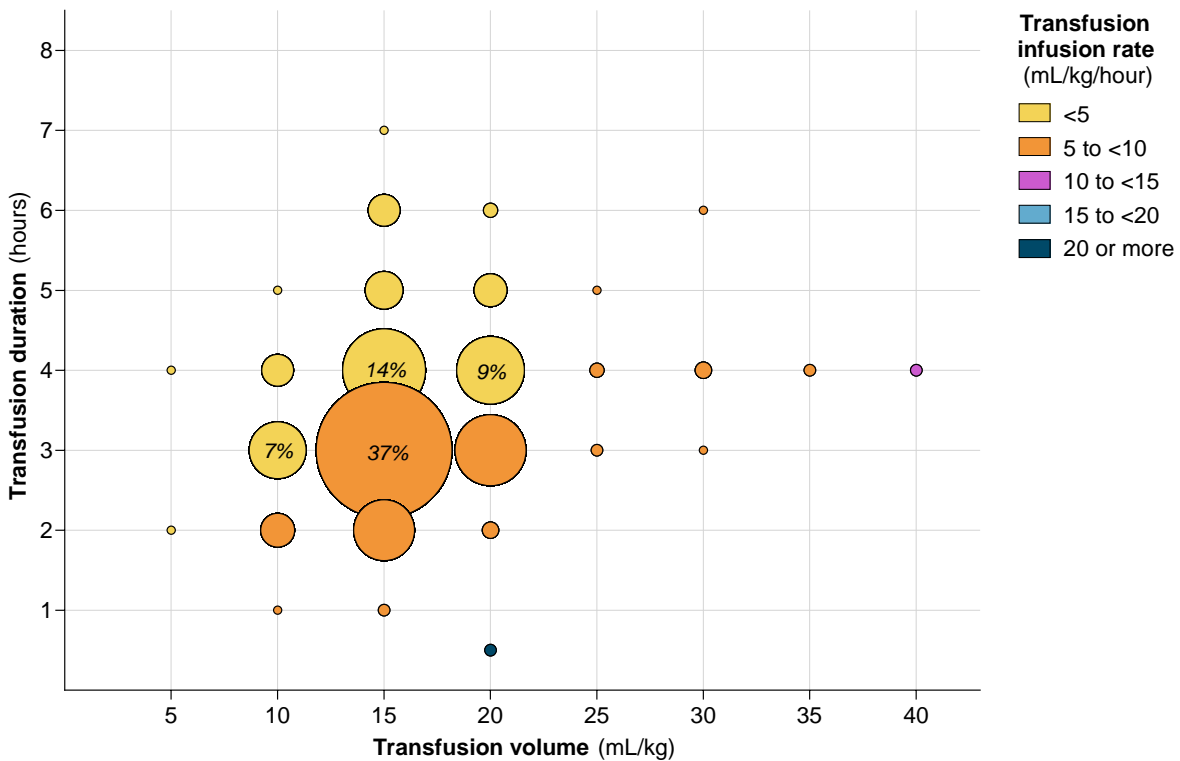
Observed prevalence rates were calculated using random effects Poisson models to pool RBC transfusion day prevalence rates from the individual centers into country subgroup estimates and subsequently to derive the overall estimate. Expected RBC transfusion day prevalence rates as predicted based on patient-mix using a logistic regression model which included the following variables: sex, multiple gestation, gestational age at birth, birth weight, postnatal day, major congenital anomalies, bleeding disorders, major bleeding, NEC, sepsis, mechanical ventilation, surgery.

**eTable 4.** Regression Coefficients for the Estimation of Expected RBC Transfusion Day Prevalence Rates

| Variable                          | Regression 1. During postnatal day 1-28 |   | Regression 2. After postnatal day 28 |   |
|-----------------------------------|---|---|--------------------------------------|---|
|                                   | Coefficient ( $\beta$ )                 | 95% CI<br>(based on asymptotic normality) | Coefficient ( $\beta$ )              | 95% CI<br>(based on asymptotic normality) |
| (Intercept)                       | -0.29                                   | -2.27, 1.68                               | -2.77                                | -5.32, -0.21                              |
| Sex*                              | -0.15                                   | -0.37, 0.08                               | -0.27                                | -0.51, -0.02                              |
| Bleeding*                         | 0.83                                    | 0.48, 1.18                                | 0.32                                 | -0.33, 0.98                               |
| Status post bleeding*             | -0.21                                   | -0.77, 0.34                               | -0.06                                | -0.62, 0.49                               |
| Postnatal age, in days            | -0.01                                   | -0.02, 0.00                               | -0.01                                | -0.02, 0.00                               |
| Surgery*                          | 0.75                                    | 0.26, 1.24                                | 0.76                                 | 0.29, 1.24                                |
| Status post surgery*              | 0.94                                    | 0.51, 1.34                                | 0.24                                 | -0.10, 0.58                               |
| Ventilation*                      | 1.41                                    | 1.09, 1.74                                | 1.43                                 | 1.09, 1.78                                |
| Status post ventilation*          | 0.75                                    | 0.42, 1.08                                | 0.46                                 | 0.12, 0.80                                |
| Sepsis*                           | 0.82                                    | 0.50, 1.13                                | 1.35                                 | 0.83, 1.86                                |
| Status post sepsis*               | 0.29                                    | -0.16, 0.73                               | 0.49                                 | 0.27, 0.75                                |
| NEC*                              | 0.34                                    | -0.31, 0.98                               | 1.00                                 | 0.52, 1.49                                |
| Status post NEC*                  | -1.94                                   | -3.11, -0.77                              | 0.16                                 | -0.57, 0.88                               |
| Gestational age at birth, in days | -0.01                                   | -0.02, 0.00                               | 0.00                                 | -0.02, 0.01                               |
| Multiparity*                      | -0.20                                   | -0.46, 0.05                               | -0.22                                | -0.51, 0.06                               |
| Congenital malformations*         | 0.09                                    | -0.47, 0.66                               | -0.22                                | -0.77, 0.33                               |
| Bleeding disorder*                | 1.33                                    | -0.17, 2.83                               | 1.58                                 | 0.84, 2.32                                |
| Birth weight, in grams            | 0.00                                    | 0.00, 0.00                                | 0.00                                 | 0.00, 0.00                                |

\*Variables: sex (0=male, 1=female), bleeding (0=no, 1=yes), status post bleeding (0=no, 1=yes), surgery (0=no, 1=yes), status post surgery (0=no, 1=yes), ventilation (0=no, 1=yes), status post ventilation (0=no, 1=yes), sepsis (0=no, 1=yes), status post sepsis (0=no, 1=yes), NEC (0=no, 1=yes), status post NEC (0=no, 1=yes), multiparity (0=no, 1=yes), congenital malformations (0=no, 1=yes), bleeding disorder (0=no, 1=yes). Definitions are available in eTable 1 in the Supplement.

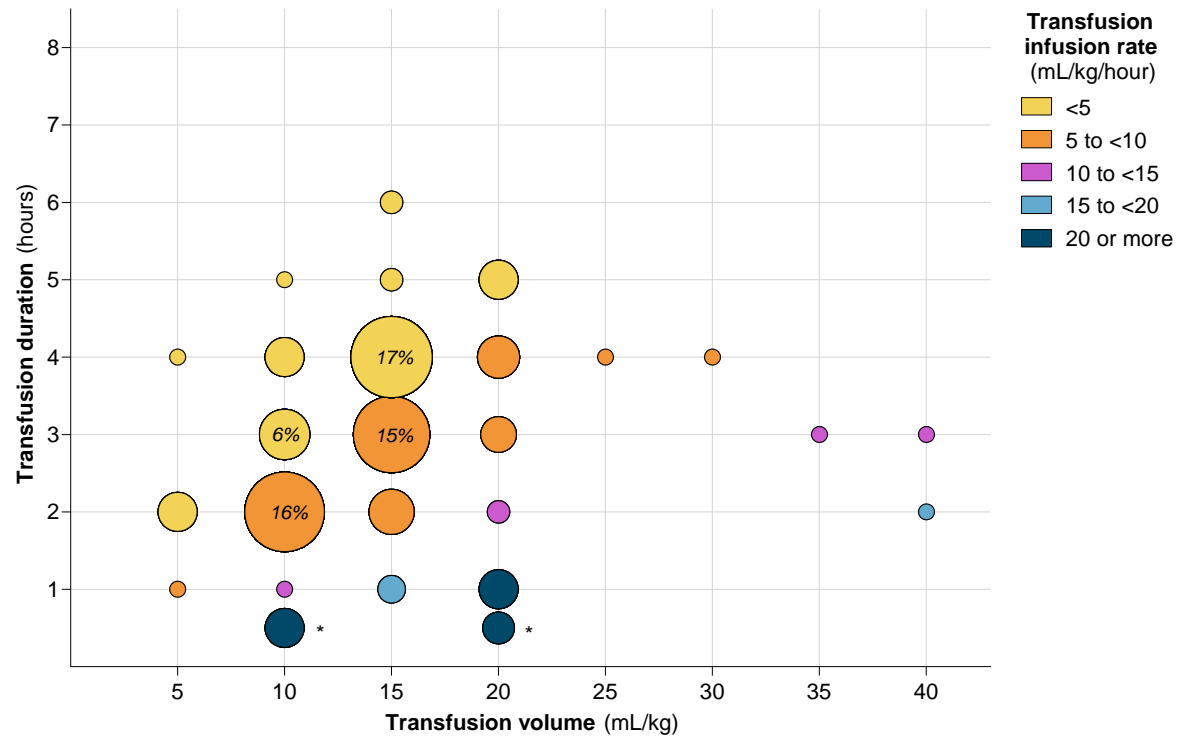
**eFigure 2.** Transfusion Volume, Duration, and Infusion Rate of 738 of 748 Transfusions Given Based on Hb Threshold



Volume and/or duration unknown in 10 transfusions. Transfusion volumes were 5 mL/kg or less in 0.3% (2/738), 10 mL/kg in 11.1% (82/738), 15 mL/kg in 63.7% (470/738), 20 mL/kg in 22.8% (168/738), 25 mL/kg in 0.8% (6/738), 30 mL/kg in 0.8% (6/738), 35 mL/kg in 0.3% (2/738), and 40 mL/kg in 0.3% (2/738). Transfusion durations were 30 minutes or less in 0.3% (2/738), 1 hour in 0.4% (3/738), 2 hours in 10.4% (77/738), 3 hours in 54.2% (400/738), 4 hours in 26.7% (197/738), 5 hours in 5.3% (39/738), 6 hours in 2.6% (19/738) and 7 hours in 0.1% (1/738) of transfusions.

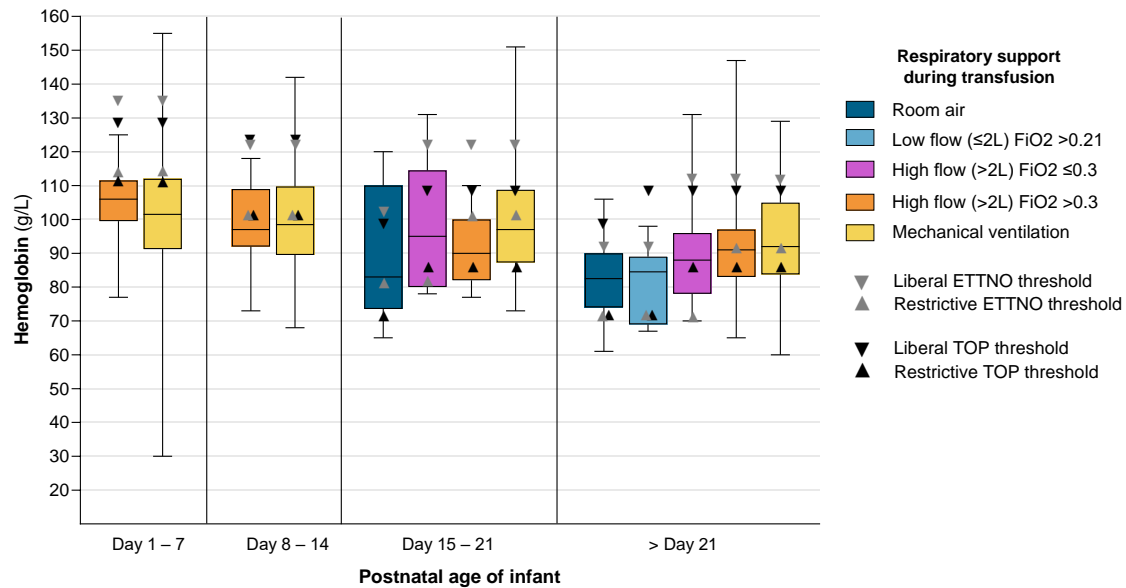


**eFigure 3.** Transfusion Volume, Duration, and Infusion Rate of 155 Transfusions Given for Bleeding, Surgery, or Any Indication Other Than Hb Threshold



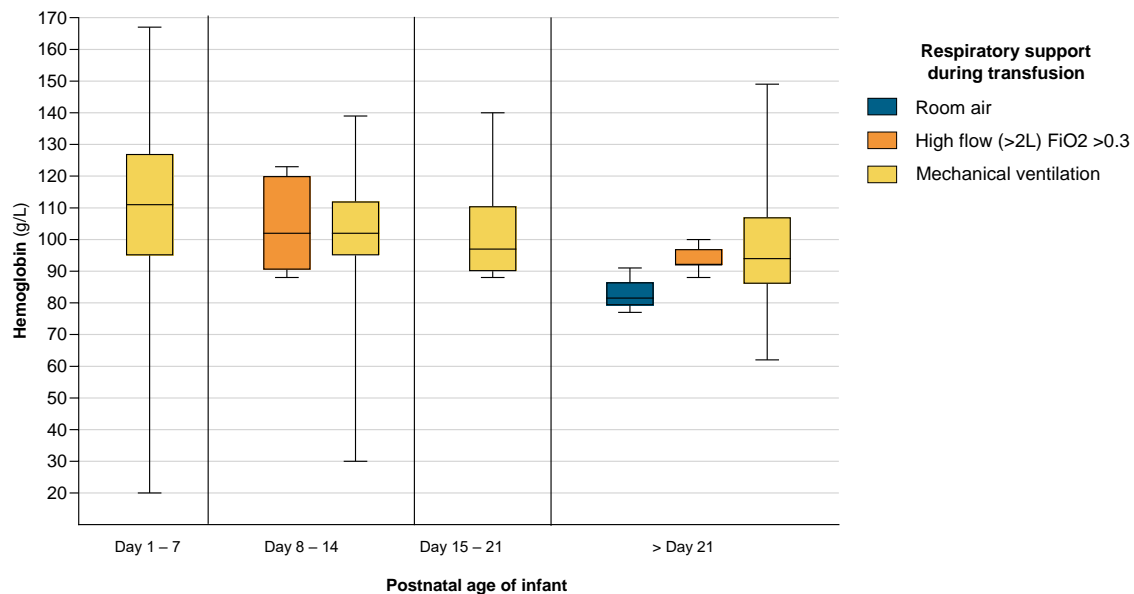
\*Transfusion duration 30 minutes or less.

**eFigure 4.** Hb Levels Prior to Transfusions Given Based on Hb Threshold



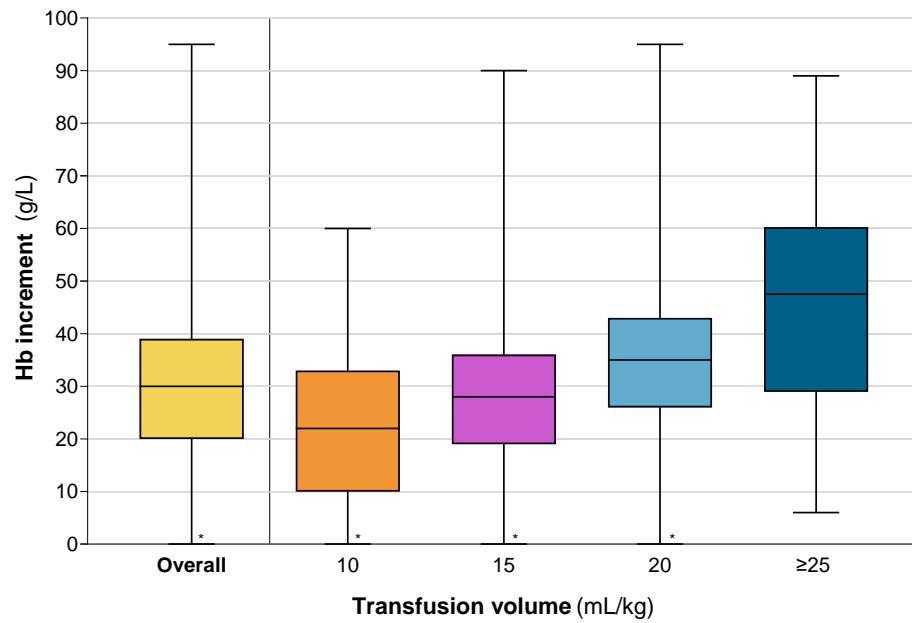
Hb levels prior to transfusion were available in 729 out of 748 transfusions given based on threshold (97.5%). High flow ( $> 2L/min$ )  $FiO_2 \leq 0.3$  or  $FiO_2 > 0.3$  by non-invasive respiratory support, including continuous positive airway pressure, biphasic intermittent positive airway pressure (synchronized or unsynchronized) and nasal high flow. Mechanical ventilation by invasive respiratory support for which the infant is intubated, including conventional mechanical ventilation and high frequency oscillation. Boxplots with fewer than 5 transfusions were omitted from the plot ( $n=13$  transfusions). The middle line of the box denotes the median, the ends of the box denote the 25<sup>th</sup> to 75<sup>th</sup> percentile, the whiskers denote the minimum to maximum range. ETTNO and TOP thresholds are available in Supplement 3. eTable 2. If respiratory support scenarios in this study could be categorized as both 'critical' or 'noncritical' according to the ETTNO trial or as both 'respiratory support' and 'no respiratory support' according to TOP trial, the highest liberal and lowest restrictive value were selected.

**eFigure 5.** Hb Levels Prior to Transfusions Given for Active Bleeding, Surgery, and Other Indications



Hb levels prior to transfusion were available in 147 out of 155 transfusions given for active bleeding, surgery, and other indications (92.9%). High flow (>2L/min) FiO2 ≤0.3 or FiO2>0.3 by non-invasive respiratory support, including continuous positive airway pressure (CPAP), biphasic intermittent positive airway pressure (synchronized or unsynchronized) and nasal high flow. Mechanical ventilation by invasive respiratory support for which the infant is intubated, including conventional mechanical ventilation and high frequency oscillation. Boxplots with fewer than 5 transfusions were omitted from the plot (n=11 transfusions). The middle line of the box denotes the median, the ends of the box denote the 25th to 75th percentile, the whiskers denote the minimum to maximum range.

**eFigure 6.** Hb Increment Stratified by Transfusion Volume



In 559 of 748 (74.7%) transfusions given based on threshold, both pre-transfusion Hb and post-transfusion Hb levels were available. Boxplots with fewer than 5 transfusions were omitted from the plot (n=1 transfusion). \*Negative Hb increment in 2 transfusions at 10mL/kg, 7 transfusions at 15 mL/kg and 1 transfusion at 20mL/kg plotted outside axis limits. The middle line of the box denotes the median, the ends of the box denote the 25th to 75th percentile, the whiskers denote the minimum to maximum range.

## eReferences

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