Professor and Head,
Department of Pediatrics, Pramukhswami Medical College, Karamsad
Associate Dean (Research Services), Charutar Arogya Mandal
National Joint Coordinator - Advanced IAP NNF NRP Program
Secretary – KMC Foundation of India
Ex-Member, Governing Body, National Neonatology Forum, New Delhi
Ex-President - National Neonatology Forum Gujarat State Chapter

James Flett Award 2012 and 2014,
Best Research Paper Award 2012 and 2015 (Excellence in Pediatrics)
Dr Arun Parikh Endowment Lecture

90+ PubMed Indexed Publications;
200+ International Research Presentations (50+ in Pediatric Academic Societies)
Reviewer for 25+ Journals incl Pediatrics, Journal of Pediatrics, Indian Pediatrics, etc
Reviewer for PAS Abstracts and Workshop

somu_somu@yahoo.com
Anemia, Transfusion and NEC
What is the Evidence?

Prof. Somashekhar Nimbalkar
Professor and Head, Department of Pediatrics, Pramukhswami Medical College
Chairman, Research Group, Charutar Arogya Mandal
Karamsad –Anand- Gujarat
President, Karamsad Academy of Pediatrics, Karamsad

@ProfSomashekhar
somu_somu@yahoo.com
https://www.linkedin.com/in/somashekhar-nimbalkar-43944b39/
Flow of the talk

• Anemia and need for transfusion
• NEC – definitions, diagnosis and pathophysiology
• Interactions between these
• History
• Current evidence
• Future studies
Anemia

- Preterm and LBWI have low levels of Hb
- Mean nadirs of 8 gm/dL in VLBW infants (birth weight: 1.0-1.5 kg) and 7 gm/dL in ELBW infants (birth weight: <1 kg)
  - Lower hemoglobin concentrations at birth
  - Frequent blood sampling
  - Low total blood volume to blood sampling ratio
  - Increased risk for other co-morbidities, and
  - Diminished capacity of the premature infant to increase EPO.
- Pathologic anemia occurs when RBC mass is inadequate to meet O2 demands
- Signs of chronic anemia can also include acidosis and tachypnea, but more often include increased resting heart rate, poor growth, decreased energy to nipple-feed, apnea, and increased need for respiratory support.
Transfusion

- Guidelines - Varying hemoglobin or hematocrit thresholds
- Stratified by postnatal age and clinical condition
- Blood loss or removal exceeds 10% of a neonate’s total blood volume

- For severe cardiopulmonary disease -- Maintain hematocrit 40%-45%
- For moderate cardiopulmonary disease -- Maintain hematocrit 30%-40%
- For major surgery -- Maintain hematocrit 30%-35%
- For infants with stable anemia -- Maintain hematocrit >20%-25%

- Severe cardiopulmonary disease defined as: requiring mechanical ventilation with >0.35 FiO2.
Transfusion Guidelines

Hct ≤32%  
Hgb <11 g/dL  
Hct <27%  
Hgb <9 g/dL  
Hct ≤24%  
Hgb ≤8 g/dL  

MAP >8 cm H₂O (CV) or MAP >14 cm H₂O (HFOV) and Fio₂ ≥0.40

Any mechanical ventilation or noninvasive ventilation and Fio₂ ≥0.40

On respiratory support (≥1 LPM and ≥0.35 Fio₂) and one of:
• ≥2 consecutive days of tachycardia (heart rate >180) or tachypnea (respiratory rate >60)
• Increase in Fio₂ by ≥0.2
• ≥2 episodes of apnea and/or bradycardia above baseline
• Undergoing surgery under general anesthesia

Hct ≤21%  
Hgb ≤7 g/dL  

Asymptomatic and absolute reticulocyte count <100,000 cells/μL (<2%)

Hct, Hematocrit; Hgb, hemoglobin; MAP, mean airway pressure; Fio₂, fraction of inspired oxygen; CV, conventional intermittent mandatory ventilation; HFOV, high-frequency oscillatory ventilation; LPM, liters per minute.
Serious Hazards of Transfusion (SHOT) – UK Data

- Rates of an adverse outcome

- 13:100,000 red cells issued for adults.
- 18:100,000 red cells issued for children younger than 18 years
- 37:100,000 for infants younger than 12 months

- Infants and neonates are disproportionately more predisposed to adverse effects from RBC transfusions than are other populations.
Transfusion risks

• Inhibiting erythropoiesis
• Associated with risks of infection
• Graft-versus-host disease (GVHD)
• Transfusion-related acute lung injury (TRALI)
• Transfusion-associated circulatory overload (TACO)
• Toxic effects of anticoagulants or preservatives.

• RBC transfusions have been reported to be associated with an increased risk of death, NEC, extension of IVH, or transient increase in respiratory support; however, high-quality evidence for a causal relationship with any of these adverse events is lacking.

• Neonatal transfusions can result in increases in proinflammatory cytokines (e.g., IL-1β, IL-8, IFN-γ, IL-17, MCP-1, IP-10, ICAM-1), potentially increasing or worsening morbidities associated with prematurity.
Low targets vs High Targets

• Current evidence suggests that a restrictive (low) transfusion threshold rather than a liberal (high) threshold (hematocrit 35%-40%) results in less exposure to transfusions, with no increase in mortality or serious morbidity.

• In developed countries, the number of transfusions given has decreased from an average of 7 in 1980s to 2 transfusions per infant in 2009

• In long-term follow-up evaluations, cognitive function based on developmental assessment and MRI outcome were better in the low-hematocrit-target group.

• A systematic review of the literature that included both RCTs & nonrandomized studies reported no differences in harmful outcomes (e.g., mortality, NEC, BPD, IVH, ROP) between restrictive and liberal transfusion strategies
Study of 4 NICUs


- Unit A and B had the following policies in place: delayed cord clamping or cord milking at very-low-birth-weight (VLBW) deliveries; drawing initial NICU blood tests of VLBW neonates using otherwise discarded fetal blood in cord/placenta; written guidelines for darbepoetin use in VLBW neonates; written guidelines to limit phlebotomy losses of VLBW neonates.

- Unit C had the following policies in place: delayed cord clamping or cord milking at VLBW deliveries; drawing initial NICU blood tests of VLBW neonates using otherwise discarded fetal blood in cord/placenta.

- Unit D had no policies in place.
NEC

• NEC is a spectrum of conditions with a similar outcome—necrosis of the intestine.

• Chain of events that leads to intestinal necrosis in a term infant with intestinal ischemia caused by a congenital heart lesion is very different when compared to a 6-week-old infant who was born at 24 weeks of gestation.

• “Classic” form of NEC peaks in preterm infants at a similar corrected GA and is most often accompanied by clinical deterioration and clear signs of intestinal inflammation, including abdominal distension, periumbilical erythema, bloody stools, elevation of nonspecific inflammatory markers, and distinctive radiologic changes such as pneumatosis intestinalis and portal venous gas.
Time and Pathophysiologic Risk Factors

- The time from birth to the onset of NEC is inversely proportional with Gestational Age (GA), with the more premature infants developing NEC at a later postnatal age, and less preterm infant developing it earlier in life.

- Development of NEC seems to reach a peak around 29-32 weeks postmenstrual age which seems to be associated with particular stages in the development of the immune response, mesenteric vasculature of the GI tract.

- Prematurity
  - Intestinal dysbiosis
  - Impaired mucosal defense mechanisms; Altered immune response
  - Altered mucosal development with subsequent increase in permeability
  - Formula feeds, Medications like antibiotics and H2-blockers
  - Altered gut perfusion; Transfusion associated gut injury
  - Dysmotility
Pathophysiology of Necrotizing Enterocolitis

- Prematurity
  - Immature gut
  - Immature immune system
  - Formula feedings
  - Antibiotics

- NEC rates
- 29-32 weeks
- Gestational age

- TLR4
- TLR9
- Paneth cells

- Dysbiosis
- Inflammation

- Vasoconstriction
- Vasorelaxation
- eNOS

- Ischemia

- Human milk

- Necrotizing enterocolitis
Clinical Signs before diagnosis of NEC

Low birth weight and gestational age, ethnicity, presence of sepsis, hypotension requiring vasopressor support, and outborn status as high to moderate quality clinical risk factors for NEC.

Need for assisted ventilation, birth via Cesarean section, premature rupture of membranes, small for gestational age, and surfactant administration have been identified as moderate quality factors determining risk for NEC.

Gephart et al. Changing the paradigm of defining, detecting, and diagnosing NEC: Perspectives on Bell’s stages and biomarkers for NEC. Seminars in Pediatric Surgery, 2018-02-01, Volume 27, Issue 1, Pages 3-10
## Modified Bell Staging in Necrotizing Enterocolitis

<table>
<thead>
<tr>
<th>BELL STAGE</th>
<th>CLINICAL</th>
<th>RADIOGRAPHIC</th>
<th>GASTROINTESTINAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Apnea and bradycardia, temperature instability</td>
<td>Normal gas pattern or mild ileus</td>
<td>Gastric residuals, occult blood in stool, mild abdominal distention</td>
</tr>
<tr>
<td>IIA</td>
<td>Apnea and bradycardia, temperature instability</td>
<td>Ileus gas pattern with ≥1 dilated loops and focal pneumatosis</td>
<td>Grossly bloody stools, prominent abdominal distention, absent bowel sounds</td>
</tr>
<tr>
<td>II B</td>
<td>Thrombocytopenia and mild metabolic acidosis</td>
<td>Widespread pneumatosis, ascites, portal venous gas</td>
<td>Abdominal wall edema with palpable loops and tenderness</td>
</tr>
<tr>
<td>III A</td>
<td>Mixed acidosis, oliguria, hypotension, coagulopathy</td>
<td>Prominent bowel loops, worsening ascites, no free air</td>
<td>Worsening wall edema, erythema and induration</td>
</tr>
<tr>
<td>III B</td>
<td>Shock, deterioration in laboratory values and vital signs</td>
<td>Pneumoperitoneum</td>
<td>Perforated bowel</td>
</tr>
</tbody>
</table>

A little History


Agwu JC, Narchi H. In a preterm infant, does transfusion increase the risk of necrotizing enterocolitis? Arch Dis Child 2005;90:102–103


- Chart review to determine relation b/w elective PRBC transfusions & occurrence of NEC

- Comparison b/w NEC patients with prior history of immediate blood transfusion (within 48 h of onset of symptoms) & those NEC patients w/o prior history of imm blood transfusion.

- Transfusion-associated NEC group developed presenting signs within 22.5 hour (median, 19; range, 12 to 38) of a PRBC transfusion at mean age of 32.7 days

- Non–transfusion-associated NEC group (n=11) had onset of NEC at a mean age of 12.7 days (p<0.05) after 185.91 hours (median, 180; range, 96 to 312; p<0.02) of a transfusion.

- Study highlighted a subset of stable, growing, premature neonates who developed a fulminant form of NEC following PRBC transfusion for symptomatic anemia.

- Whether risk of “transfusion-associated” NEC is higher in infants with lower hematocrits and advanced postnatal age.
- Retrospective comparison of NEC patients & controls born at <34 weeks GA.

- Frequency of RBC transfusions was similar in NEC patients (47/93, 51%) & control patients (52/91, 58%).
- Late-onset NEC (>4 weeks of age) was more frequently associated with a history of transfusion(s) than early onset NEC (adjusted OR, 6.7; 95% CI, 1.5 to 31.2; P = .02).

- A history of RBC transfusions within 48-hours before NEC onset was noted in 38% of patients, most of whom were extremely low birth weight infants.
- Relationship between RBC transfusions and NEC requires further evaluation in extremely low birth weight infants using a prospective cohort design.
RBC Transfusions and NEC

• In a retrospective chart review, Stritzke reported association between NEC & transfusion, with 5.5% of patients receiving transfusion 2 days prior to the diagnosis of NEC.

• Another retrospective chart review found 6% absolute difference with increased NEC in infants fed vs. not fed during transfusion, but this difference was not statistical significant.

• Other studies did not find any associations between transfusion and NEC

• 2 published studies that found transfusions to actually be protective
RBC Transfusions and NEC – meta-analysis

- Meta-analysis of 11 retrospective case-control studies & one cohort study showed that recent exposure to transfusion was associated with NEC in neonates.


- Neonates who developed NEC were at overall higher risk of mortality

- Updated meta-analysis which addresses the same question from 17 observational studies showed no temporal relationship between RBC transfusions and NEC.

- Effect size for this relationship differed between matched case-control studies (odds ratio [OR] 1.20, 95% CI 0.58–2.47; p = 0.63) and differed from those reported in cohort studies (OR 0.51, 95% CI 0.34–0.75; p ≤0.01).


- Contrast in results based on the study type and methodology underscores the need for high-quality prospective evidence in the area
RBC Transfusions, Mucosal Injury and Inflammation

• A preexisting intestinal injury in at least 54% of all transfusion cases and in 75% of NEC cases that increased to 75 and 100% respectively post-transfusion, using urinary iFABP/Cr as a surrogate for intestinal injury. Also feeding during transfusion did not seem to influence the outcome.

• A prospective observational study in infants less than 32 weeks gestation, that measured inflammatory markers 2 hours before and 2, 12, 24 and 48 hours after PRBC transfusion did show an increase in IL-1β, IL-8, INF-γ, IL-17, MCP-1, IP-10 at different times post-transfusion, suggesting an inflammatory reaction that could contribute to transfusion-associate gut injury
A final overall quality of “very low” for the evidence for an association between transfusions and necrotizing enterocolitis.

Pooled outcome of NEC for observational/case control studies was an odds ratio of 1.13 (95% CI: 0.99–1.29) when TANEC was defined as occurring within 48 hours of transfusion.

For NEC occurring at anytime post-transfusion, the pooled OR was 1.95 (1.60–2.38).

Conversely, the pooled outcome of NEC for the RCT data had an odds ratio of 0.6 (0.3, 1.21) with NEC being less frequent in the liberal transfusion group compared to the restrictive transfusion group.
Is it the Anemia or is it the Transfusion?

• Patel et al., in a prospective study, showed that among VLBW infants, severe anemia (defined as hemoglobin<8 g/dl), instead of RBC transfusions, is associated with an increased risk (six fold) of NEC.

• Each 1g/dL decrease in lowest measured hemoglobin in a given week was associated with a 65% increase in the risk of NEC (p< 0.01). Between 49 and 90 days of age, infants with NEC, compared to those without NEC, tended to have a lower hemoglobin (mean difference = −1.5g/dL, p = 0.06).


• Need for further studies to evaluate whether preventing severe anemia is more important than minimizing RBC transfusions.

• Goobie et al. recently suggested an association between preoperative anemia and postoperative mortality in neonates

The emphasis on anemia was based on several emerging lines of evidence

(a) NEC has been reported in patients with anemia related to diverse neonatal conditions such as glucose-6-phosphate dehydrogenase deficiency, hemolytic disease of the newborn, and twin-to-twin transfusion syndrome


(b) TANEC is usually a late event seen beyond 4 weeks of postnatal age, when these infants are usually anemic. (Josephson, C. D. et al. J. Pediatr. 157, 972–978 (2010).)

(c) Intestinal injury is also seen in other critically ill infants such as those undergoing treatment with cardiopulmonary bypass or ECMO, particularly when they receive top-up transfusions to treat severe anemia (Huybregts, R. A. et al. Anesth. Analg. 109, 331–339 (2009))

(d) Neonates with transfusion-associated NEC may have had one or more previous RBC transfusions.

- C57BL/6 mouse pups rendered anemic by timed phlebotomy and then given RBC transfusions develop NEC-like intestinal injury within 12–24 h.
- Anemic intestine is infiltrated by inflammatory macrophages, which are activated in situ by RBC transfusions via a Toll-like receptor (TLR)-4-mediated mechanism and cause bowel injury.
- Intestinal injury worsens with increasing severity and the duration of anemia prior to transfusion, indicating a need for the re-evaluation of current transfusion guidelines for premature infants.

**CONCLUSIONS**

- Severe anemia in the murine neonate leads to the development of a low-grade inflammatory state in the intestine with prominent macrophage infiltration.
- Subsequent RBC transfusions activate these macrophages and causes NEC-like intestinal injury.

Feeding and transfusion-related NEC

- Mostly before after or Quality Improvement studies.


- Talavera showed a decrease in NEC incidence from 8% to 3.1% (p< 0.001), with continued decline to 0.9% after additional interventions over a 3 year period as part of the QI effort. It is likely that any benefit from withholding feedings is multi-factorial.

- Have shown benefit for withholding feeds but the design of studies do not give any indication of what needs to be done.
Stopping enteral feeds for prevention of transfusion-associated NEC in preterm infants (Review)

Yeo KT, Kong JY, Sasi A, Tan K, Lai NM, Schindler T.
Cochrane Database of Systematic Reviews 2019, Issue 10. Art. No.: CD012888

• Randomised controlled trial evidence is insufficient to show whether stopping feeds has an effect on the incidence of subsequent NEC or death.

• Only one RCT involving 22 preterm infants was eligible for inclusion in the review.

• Primary objective of this trial was to investigate changes in mesenteric blood flow, & no cases of NEC were reported in any of the infants included in the trial.

• Large, adequately powered RCTs are needed to address this issue.
Does a Liberal Red Blood Cell Transfusion Strategy Improve Neurologically-Intact Survival of Extremely-Low-Birth-Weight Infants as Compared to a Restrictive Strategy

Neonatal Research Network – 18 academic centers

**TRANSFUSION OF PREMATURES (TOP) TRIAL**
Objectives and Aims

• Two previous trials -the PINT (Prematures In Need of Transfusion; J Pediatr. 2006;149:301-7) and its follow-up PINT-Outcome Study (PINTOS) (Pediatrics 2009; 123: 207-13) and the Iowa trial (Pediatrics 2005; 115: 1685-91) and its follow up study (Arch Pediatr Adolesc Med. 2011 ;165(5):443-50) raise important but contradictory implications for CNS injury and development.

• Primary objective of the TOP trial is to determine whether higher hemoglobin thresholds for transfusing ELBW infants resulting in higher hemoglobin levels lead to improvement in the primary outcome of survival and rates of neurodevelopmental impairment (NDI) at 22-26 months.

• Specific Aim 2 (Short term to NICU Discharge)
  (e) No. of transfusions, No. of donor exposures by RBC donors or other blood product.
  (h) Episodes of NEC of Bell stage 2 or higher, and time to full feeds.
Effects of Transfusion Thresholds on Neurocognitive Outcome of Extremely Low Birth-Weight Infants (ETTNO)

ETTNO
Primary outcome, death or neurodevelopmental impairment, will be determined at 24 months of age, corrected for prematurity.

Nine hundred and twenty infants of 400–999 g birth weight will be randomized to restrictive or liberal transfusion trigger thresholds between 48 and 72 h of life, stratified by center and birth weight (400–749 g/750–999 g).

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<tr>
<th>Time after</th>
<th>Hematocrit, %</th>
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Individual patient data meta-analyses to
(a) verify the effect of different levels of transfusion trigger thresholds on neurodevelopmental outcome and
(b) analyze the impact of transfusion triggers on less frequently observed complications of prematurity, such as severe retinopathy, severe chronic lung disease, necrotizing enterocolitis and others.

(4) more than 6 apneas that require stimulation per 24 h, or more than 4 desaturations to \( \text{SpO}_2 < 60\% \) per 24 h despite methylxanthines and continuous positive airway pressure and (5) acute sepsis or acute necrotizing enterocolitis requiring inotropic or vasopressor support.
WHEAT trial

- **WithHolding Enteral feeds Around packed red cell Transfusion to prevent necrotising enterocolitis in preterm neonates.**
- Multi-centre, randomised point of care trial.
- Running in neonatal units in Northwest London and around Birmingham
- [https://www.npeu.ox.ac.uk/wheat](https://www.npeu.ox.ac.uk/wheat)
- 179 out of 250 recruited
- Medical Research Council (MR/N005405/1) funding
Summary

• “Transfusion/anemia-associated NEC” appears to be a plausible clinical entity

• Cautious interpretation of data (Bias, confounding variables, reverse causation)

• Large, prospective, multi-center trials are ongoing

• Clinical and pre-clinical studies to understand the potential interaction between anemia and RBC transfusion on the outcome of NEC are needed

• Strategies to decrease RBC transfusions, such as use of rEPO or synthetic, longer-acting darbepoietin
Thank You