

Red Blood Cell Transfusion Exposure Is Linked to Lower CD8+ T-Cell Counts in Very Low Birthweight Infants

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Abstract

Background

Very low birthweight (VLBW) infants frequently require red blood cell (RBC) transfusions during early postnatal life. Emerging evidence suggests that transfusions may modulate immune responses, but its impact on immune development remains incompletely understood.

Methods

136 VLBW infants participating in the prospective “Immune Regulation of the Newborn Study” (IRON study) were analyzed during the first 42 postnatal days with serial immune phenotyping. Infants were stratified by RBC transfusion exposure. Group differences were assessed using Mann-Whitney U tests at each postnatal week and multivariable linear regression models were used to evaluate the association between RBC transfusion and log-transformed CD8 + T-cell counts. For a dose-dependent effect, log-transformed CD8 + T-cell counts were regressed on the number of transfusions and on the cumulative transfusion burden.

Results

Transfused infants were more premature and had higher morbidity. Longitudinal analysis demonstrated persistently lower CD8 + T-cell counts in transfused infants. At day 42, RBC transfusion was independently associated with reduced CD8 + T-cell levels in multivariable regression ($\beta = -0.312$, 95% CI -0.578 to -0.045 , $p = 0.022$), corresponding to approximately 27% lower CD8 + T-cell counts among transfused infants. Across all six weekly windows, a higher number of transfusions is consistently associated with lower CD8 + T-cell counts reaching statistical significance in week 2 and week 5.

Conclusions

RBC transfusion exposure in VLBW infants is independently associated with altered adaptive immune development, particularly reduced CD8 + T-cell levels over time. Our findings indicate an RBC-specific effect supporting the concept of RBC transfusion-related immunomodulation in preterm infants and warrant further investigation.

Background

Preterm birth remains a major global health challenge and is associated with increased neonatal mortality and substantial long-term morbidity [1–3]. Very low birthweight (VLBW) infants (< 1,500 g) are particularly vulnerable because developmental immaturity of multiple organ systems intersects with inflammatory and oxidative stress pathways that contribute to major complications such as prematurity-

associated lung disease (PLD) and brain injury [4, 5]. These infants frequently require intensive care interventions that may further modulate immune and inflammatory responses. Among these interventions, transfusion of blood products, particularly red blood cells (RBC), is common during the first weeks of life [6, 7]. However, transfusion therapy is not biologically inert. Data is lacking about the immunological consequences and potential links to short-term clinical outcomes in VLBW infants [8–10].

In older patient populations, transfusion has been associated with transfusion-related immunomodulation (TRIM), encompassing both immunosuppressive and proinflammatory effects [11, 12]. Proposed mechanisms include host, environmental, and treatment-related factors leading to activation of inflammatory and coagulation pathways through transfusion [11]. In recipients with pre-existing inflammatory priming, transfusion has been associated with increased levels of circulating proinflammatory cytokines and endothelial activation, consistent with a “multiple-hit” model in which baseline inflammation amplifies transfusion-triggered responses [13, 14].

This paradigm may be particularly relevant in VLBW infants, who frequently exhibit inflammatory activation due to antenatal and postnatal exposures [4]. Emerging neonatal data indicate that RBC transfusions may be followed by increases in proinflammatory cytokines and markers of endothelial activation, with potential dose-dependent effects after repeated transfusions [10, 15, 16]. The immune system of very preterm infants is developmentally constrained and characterized by reduced adaptive immune function and limited regulatory capacity, resulting in increased susceptibility to infection and dysregulated inflammatory responses [17, 18].

However, data integrating immune cell phenotyping with transfusion exposure in VLBW infants remain limited. We therefore investigated the immunologic and clinical effects of RBC transfusions in VLBW infants during the first 42 postnatal days. We hypothesized that transfusion exposure within this period would be associated with distinct alterations in immune cell profiles.

Methods

Study design and setting

This study is a retrospective, single-center observational analysis conducted at the University Hospital Schleswig-Holstein, Campus Lübeck. The analysis leverages data and serial immune phenotyping obtained within the prospective “Immune Regulation of the Newborn Study” (IRON study), initiated in 2014, to investigate immunoregulatory mechanisms and inflammatory effects in VLBW infants.

Within the IRON study, blood samples were obtained serially during hospitalization with a focus on early postnatal adaptation. Additional blood draws occurred as clinically indicated (e.g., suspected infection, surgery, diagnostic procedures).

Study population

Eligible participants were VLBW infants born before 37 weeks of gestation and delivered between January 2014 and December 2020. To specifically address the immunological effect of RBC transfusions and to avoid heterogeneity introduced by exposure to multiple blood product types, the analysis cohort was restricted to infants who either received RBC transfusions only or who received no transfusion at all during the first 42 postnatal days ($n = 136$). Infants with exposure to platelet concentrates or fresh frozen plasma (FFP) were excluded from the analysis. Infants who received at least one RBC transfusion within the first 42 postnatal days were classified as transfused. RBCs were irradiated prior to administration. Detailed data on storage duration and donor exposure were not consistently available and could not be included in the present analysis. Infants with incomplete clinical or laboratory datasets were excluded.

Ethics approval and consent

The IRON study was approved by the Ethics Committee of the University of Lübeck (reference AZ 2024_222; formerly AZ 15_304). Written informed consent was obtained from parents or legal guardians for study participation and processing of clinical and laboratory data.

Laboratory methods

Flow cytometry

Immune cell populations were quantified by flow cytometry (fluorescence-activated cell sorting; FACS) using a FACSCanto system (BD Biosciences, Heidelberg, Germany). Analyses were performed with BD FACSDiva software. Cell populations were identified based on forward/side scatter characteristics and fluorescence intensity. Panel and gating: T, B, and NK cell typing included CD3⁺, CD4⁺, CD8⁺, CD19⁺, CD56⁺, and CD16⁺ populations. Whole blood was stained with two pre-formulated BD Multitest antibody panels, and erythrocytes were lysed with BD FACS Lysing Solution according to the manufacturer's instructions; absolute cell counts were determined using BD Trucount tubes. The BD Multitest 6-Color TBNK panel was used to enumerate T, B and NK cells and CD4⁺/CD8⁺ T-cell subsets [anti-CD3 (FITC, clone SK7), anti-CD16 (PE, B73.1), anti-CD56 (PE, NCAM16.2), anti-CD45 (PerCP-Cy5.5, 2D1), anti-CD4 (PE-Cy7, SK3), anti-CD19 (APC, SJ25C1) and anti-CD8 (APC-Cy7, SK1)], and the BD Multitest CD3/CD8/CD38/HLA-DR panel was used to identify activated cytotoxic T cells (CD3⁺CD8⁺HLA-DR⁺) based on HLA-DR and CD38 expression [anti-CD8 (FITC, SK1), anti-CD38 (PE, HB7), anti-CD3 (PerCP, SK7) and anti-HLA-DR (APC, L243)]. Absolute counts ($\times 10^9/L$) were derived for six lymphocyte populations. The immunophenotypic (gating) definitions and the corresponding cell populations are summarized in supplementary table 1.

Data sources and data collection

Clinical and laboratory information was extracted from electronic and paper-based patient records. General patient characteristics were obtained from discharge summaries and birth documentation. Laboratory data were retrieved from the hospital information system, and detailed respiratory and transfusion documentation was obtained from the intensive care charting system and the transfusion laboratory system. Further information is found in the Supplementary material.

Data were entered into a pseudonymized electronic database (Microsoft Excel) and transferred to IBM SPSS Statistics (26.0; IBM Corp., USA) for analysis. Data processing complied with EU GDPR and German data protection regulations. Identifiers were removed and replaced by study case numbers.

Statistical analysis

The primary aim was to compare transfused versus non-transfused VLBW infants with respect to immune cell parameters, laboratory indices, and clinical outcomes. Transfusion exposure was modeled as a time-varying, cumulative variable. At each predefined time point, infants were classified as “transfused” if they had received at least one transfusion prior to or at that time point. Accordingly, exposure status could change over time, and infants contributed person-time to the non-transfused group until their first transfusion, after which they were classified as transfused for all subsequent time points.

Univariate comparisons

Because several variables were non-normally distributed, non-parametric methods were applied without formal normality testing. Continuous variables were summarized as median with interquartile range (IQR) and categorical variables as count (relative frequency in %), with 95% confidence interval (CI) where appropriate. Missing values were not imputed. Group comparisons used Mann–Whitney U tests for continuous variables and chi-square tests for categorical variables.

Multivariable models

To partially account for confounding, regression models were used for selected endpoints. Linear regression models assessed associations of transfusion exposure, gestational age, birthweight, and blood-culture proven sepsis with CD8 + T-cell concentrations. CD8 + T-cell counts showed right-skewed distributions and were log-transformed prior to regression analysis to meet model assumptions. Model assumptions, including normality of residuals and homoscedasticity, were assessed using histograms and normal probability (P–P) plots of standardized residuals. A two-sided p-value < 0.05 was considered statistically significant.

Dose-response analysis

To examine whether CD8 + T-cell counts varied with the amount of transfusion exposure, the number of RBC transfusions was derived from daily transfusion indicators. Daily indicators were summed within each of the six weekly windows to obtain the number of transfusions per window, and cumulatively up to each measurement day to obtain the cumulative transfusion burden. For each weekly window, log-transformed CD8 + T-cell counts were regressed on the number of transfusions in that window. In parallel, log-transformed CD8 + T-cell counts at each measurement day were regressed on the cumulative transfusion burden up to that day. Associations were expressed as effect ratios (exponentiated coefficients) with 95% CI. Given the sparse and right-skewed exposure distribution, Pearson correlations on the log scale were complemented by Spearman rank correlations on untransformed values as a robustness check. Weekly models with sufficient exposure were additionally adjusted for birth weight, culture-proven sepsis, intraventricular hemorrhage, necrotizing enterocolitis, and total duration of oxygen supplementation.

Results

Study cohort

A total of 454 VLBW infants born < 37 weeks' gestation between January 2014 and December 2020 were screened (Fig. 1). After exclusion of infants exposed to platelet or fresh frozen plasma transfusions and those with incomplete datasets, 136 infants who received either RBC transfusions only or no transfusion at all were included in the analysis cohort. Among these, 29 (21.3%) infants received at least one RBC transfusion during the first 42 days of life, and 107 (78.7%) did not.

Baseline characteristics differ between groups (Table 1).

Table 1

Baseline characteristics stratified by transfusion practice. Categorical variables are given as n (%) with corresponding 95% confidence interval (CI); continuous variables as median (IQR) if appropriate (#).

Variable	Transfusion n = 23	No Transfusion n = 113
Gestational age [weeks]#	25.9 (24.3–27.1)	28.6 (27.4–30.1)
Birth weight [grams]#	790 (670–920)	1200 (970–1400)
No antenatal steroids	3 (13.0 [3.8–30.9])	18 (15.9 [10.1–23.5])
No Application of Surfactant	0	3 (2.7 [0.8–6.9])
Culture-proven sepsis	9 (47.4 [26.6–68.8])	8 (7.2 [3.5–13.1])
Clinical sepsis	9 (45.0 [25.1–66.2])	44 (38.9 [30.3–48.1])
Intracerebral hemorrhage	6 (26.1 [11.7–46.1])	11 (9.7 [5.3–16.2])
Spontaneous intestinal perforation	1 (4.3 [0.5–18.6])	3 (2.7 [0.8–6.9])
Necrotizing enterocolitis	0	2 (1.8 [0.4–5.6])
Bronchopulmonary dysplasia	4 (17.4 [6.2–36.2])	9 (8.0 [4.1–14.2])
Retinopathy of prematurity	14 (60.9 [40.6–78.6])	35 (31.0 [23.0–39.9])

Transfused infants have a lower median gestational age (25.9 [24.3–27.1] vs. 28.6 [27.4–30.1] weeks) and a lower median birth weight (790 [670–920] vs. 1200 [970–1400] grams) than non-transfused infants. Several neonatal morbidities occur more frequently in the transfused group, including culture-proven sepsis, intracerebral hemorrhage, bronchopulmonary dysplasia, and retinopathy of prematurity. The cumulative distribution of transfusions across the first 6 postnatal weeks is shown in Fig. 2.

Immune cell profiles

Univariate analyses

Longitudinal trajectories of immune cell populations according to transfusion exposure

Longitudinal analysis of immune cell trajectories over the first 6 weeks of life reveals distinct patterns between transfused and non-transfused VLBW infants in CD8 + T cell counts (Fig. 3, Supplementary Table 2). While CD8 + T cell levels increase over time in both groups, transfused infants exhibit persistently lower CD8 + T cell counts with statistically significant differences emerging from week 2.

In contrast, other immune cell populations show no consistent or sustained differences between groups over time (Supplementary Fig. 1, Supplementary Table 2).

Multivariable models

Model diagnostics confirm that assumptions of linear regression are met. Residual plots demonstrate no evidence of heteroscedasticity, and variance inflation factors remain well below commonly accepted thresholds of the variance inflation factor ($VIF < 5$), excluding relevant multicollinearity. Standardized residuals do not exceed ± 3 , and Cook's distance values remain below 1.

In multivariable linear regression analyses with log-transformed CD8 + T cell counts as the dependent variable, the association between RBC transfusion and CD8 + T cell levels differ by time point (Fig. 4). At day 28, the overall model is not statistically significant ($R^2 = 0.108$, adjusted $R^2 = 0.048$, $p = 0.139$; $n = 65$). RBC transfusion shows a trend toward lower CD8 + T cell counts that does not reach formal statistical significance ($\beta = -0.385$, 95% CI -0.840 to 0.071 , $p = 0.097$). Birth weight, gestational age, and culture-proven sepsis are not significantly associated with CD8 + T cell levels at this time point. At day 42, the multivariable model is statistically significant ($R^2 = 0.099$, $p = 0.030$; $n = 107$). RBC transfusion is independently associated with lower CD8 + T cell counts ($\beta = -0.312$, SE = 0.134 , standardized $\beta = -0.247$, 95% CI -0.578 to -0.045 , $p = 0.022$). On the ratio scale, this corresponds to an approximately 27% reduction in CD8 + T cell levels among RBC-transfused infants. Birth weight is independently and positively associated with CD8 + T cell counts (standardized $\beta = 0.311$, $p = 0.027$), whereas gestational age ($p = 0.078$) and culture-proven sepsis ($p = 0.771$) are not independently associated.

Dose-response relationship

To assess whether CD8 + T-cell counts varied with the amount of transfusion exposure, we modeled the number of RBC transfusions received within each postnatal week and the cumulative transfusion burden up to each measurement day (Supplementary Fig. 2, Supplementary Table 3). In our cohort, transfusion exposure is sparse: between 4 and 14 infants are transfused within any single week, and a small number receive multiple transfusions within one week (up to eight). Across all six weekly windows, a higher number of transfusions is consistently associated with lower CD8 + T-cell counts (all point estimates < 1). The association reaches statistical significance in week 2 (effect ratio 0.50, 95% CI 0.37–0.68, $p < 0.001$; $R^2=0.22$; $n = 74$) and week 5 (effect ratio 0.66, 95% CI 0.50–0.87, $p = 0.004$; $R^2=0.14$; $n = 58$). The remaining windows show associations in the same direction that do not reach significance. The week-5 association is confirmed by rank correlation (Spearman $\rho=-0.36$, $p = 0.006$), whereas the week-2 association, although strong on the log-linear scale (Pearson $r = -0.47$, $p < 0.001$), is only a non-significant trend by rank (Spearman $\rho=-0.20$, $p = 0.088$), indicating sensitivity to a few heavily transfused infants.

Using cumulative transfusion burden, log-transformed CD8 + T-cell counts decrease with increasing cumulative exposure at every measurement day from day 14 onward (day 14: effect ratio 0.82 per

additional transfusion, 95% CI 0.76–0.89, $p < 0.001$, $R^2=0.25$; day 21: 0.93, $p = 0.017$; day 28: 0.90, $p = 0.005$; day 35: 0.96, $p = 0.006$; day 42: 0.96, $p = 0.017$). The diminishing per-transfusion magnitude over time reflects the growing cumulative count, while the direction remains inverse throughout. In weekly models additionally adjusted for birth weight, culture-proven sepsis, intraventricular hemorrhage, necrotizing enterocolitis, and oxygen-supplementation duration, the transfusion-count estimates attenuate and are no longer statistically significant (e.g., day 28: $\beta=-0.368$, $p = 0.245$).

Discussion

In this study of VLBW infants, RBC transfusion exposure during the first 42 days of life is independently associated with lower CD8 + T cell counts, suggesting an alteration in postnatal adaptive immune development. This observation is biologically plausible in the context of neonatal immune ontogeny and transfusion-related immunomodulation. Preterm infants exhibit an immature adaptive immune system characterized by limited T-cell function, reduced proliferative capacity, and altered CD4/CD8 balance in early life [19–22]. During the first weeks after birth, immune cell populations undergo rapid developmental changes, making this period particularly vulnerable to external perturbations [20, 23, 24]. In this setting, transfusion may not simply induce transient immunologic changes but may instead modify developmental trajectories of adaptive immune subsets, particularly cytotoxic T-cells. The delayed emergence of significant differences in our data supports this concept of a cumulative or time-dependent effect, rather than an immediate post-transfusion response. Blood transfusion is increasingly recognized as an immunologically relevant [25] intervention. The concept of transfusion-related immunomodulation (TRIM) describes a spectrum of effects ranging from immune activation to immune suppression, depending on host and environmental factors. In preterm infants, several studies have demonstrated that red blood cell transfusions are associated with increases in proinflammatory cytokines, including IL-1 β , IL-8, TNF- α , and MCP-1, as well as markers of endothelial activation such as soluble ICAM-1 and macrophage migration inhibitory factor [15, 16, 26, 27], with the magnitude of these responses increasing with repeated and cumulative transfusion exposure [15, 16]. By modeling transfusion exposure as a cumulative variable in our study, our analysis supports a biologically plausible interpretation of a dose-dependent effect on immune development. A higher number of RBC transfusions is associated with lower CD8 + T-cell counts, and cumulative transfusion burden shows a graded inverse association with CD8 + T-cell counts from the second week of life onward, consistent with a cumulative rather than transient immunomodulatory effect. However, these signals should be interpreted with caution. Transfusion exposure within individual weeks is sparse, and for some windows – most notably week 2 – the log-linear association is driven by a small number of heavily transfused infants and is not confirmed by rank-based testing. In addition, the weekly dose-response estimates attenuate after adjustment for illness-severity covariates, underscoring that confounding by indication cannot be excluded and that the dose-response relationship remains hypothesis-generating.

From a mechanistic perspective, the observed reduction in CD8 + T cell counts may reflect a shift in the balance between innate and adaptive immunity. Transfusion-associated inflammatory activation may preferentially engage innate pathways, while simultaneously impairing or delaying the expansion of

adaptive cytotoxic T-cell populations [10]. Further mechanisms that may contribute to the observed peripheral reduction are worth to be discussed. First, delayed adaptive maturation is a hallmark of extreme prematurity: preterm infants have lower T-cell receptor excision circle (TREC) levels at birth than term infants, and TREC values rise with increasing gestational age, indicating that thymic output is constitutively reduced in the most immature infants ([28]). A lower baseline thymic output is compatible with a slower kinetic expansion of cytotoxic T-cell populations during the early postnatal period, which may be accentuated in infants exposed to additional postnatal stressors. Second, peripheral consumption through ongoing antigenic stimulation – for example by recurrent infectious episodes, prolonged mechanical ventilation, or transfusion-associated alloantigen exposure – may further contribute to the observed reduction. These mechanisms may exhaust circulating CD8 + T cells via activation-induced cell death and progression toward terminal exhaustion [29, 30]. Redistribution, delayed maturation, and consumption are not mutually exclusive and may act in concert to produce the persistently reduced peripheral CD8 + T-cell counts observed in our cohort. Future studies integrating further immunophenotyping (e.g. TRECs), and activation profiles would help disentangle these contributions.

This study has important limitations. One limitation is the potential for confounding by indication. Importantly, transfusion exposure in this cohort likely reflects underlying illness severity rather than an isolated intervention. Despite adjustment for gestational age, birth weight, and sepsis, residual confounding by factors such as respiratory support intensity, inflammatory burden, and overall clinical instability is highly likely. Transfused infants in our cohort are more premature and had higher rates of sepsis and other complications, all of which are independently associated with altered immune development [4, 31]. Although multivariable models adjusted for key covariates, substantial residual confounding related to overall illness severity, inflammatory burden, and intensity of clinical support is likely. Therefore, the observed associations should be interpreted cautiously and considered hypothesis-generating rather than causal. Future studies should apply more advanced causal inference approaches to better address confounding by indication. A further limitation of our study is the lack of assessment of activation and functional markers of T cells such as CD38, HLA-DR, PD-1, CD45RA/RO, or intracellular cytokine production (e.g., IFN- γ) [32, 33]. Our analysis is limited to quantification of immune cell populations and does not provide insight into activation status, differentiation, or functional capacity of CD8 + T cells. As transfusion-related immune modulation may primarily affect functional properties rather than absolute cell numbers, the observed differences in CD8 + T cell counts may underestimate the true extent of immunologic alterations. Future studies incorporating activation markers and functional assays are needed to further elucidate these mechanisms.

Despite these limitations, our findings suggest that RBC transfusion exposure is linked to relevant alterations in adaptive immune development in VLBW infants. The RBC-specific reduction in CD8 + T cell levels highlight the potential importance of transfusion practice for neonatal immune maturation. These data support ongoing efforts to optimize transfusion strategies and to better understand the immunologic consequences of common neonatal interventions.

Conclusion

In VLBW infants, RBC transfusion exposure is independently associated with altered postnatal immune development, particularly a reduction in CD8 + T cell counts. These findings support the concept of RBC transfusion-related immunomodulation in the developing immune system. Further studies integrating longitudinal immune profiling, functional T-cell assays, and detailed transfusion characteristics are needed to clarify causality and underlying mechanisms.

Abbreviations

BPD Bronchopulmonary dysplasia

CI Confidence interval

FFP Fresh frozen plasma

IQR Interquartile range

IRON Immune Regulation of the Newborn Study

OR Odds ratio

RBC red blood cell

TRIM transfusion-related immunomodulation

VIF Variance inflation factor

VLBW Very low birthweight

WBC White blood count

Declarations

Competing Interest

None declared

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contribution

NO Acquisition, Conceptualization, Data curation, Preparation, creation and presentation of the published work, specifically writing the initial draft

MIF Conducting research and investigation process

KF Conducting research and investigation process

JP Management and coordination responsibility for the research activity planning and execution, Provision of study materials

MD Application of statistical techniques to analyze or synthesize study data

CH Methodology, Management and coordination responsibility for the research activity planning and execution, Supervision

AH Methodology, Preparation, creation of the published work (critical review and revision), Application of statistical techniques to analyze or synthesize study data.

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Data Availability

The datasets for this study are available on reasonable request. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Figures

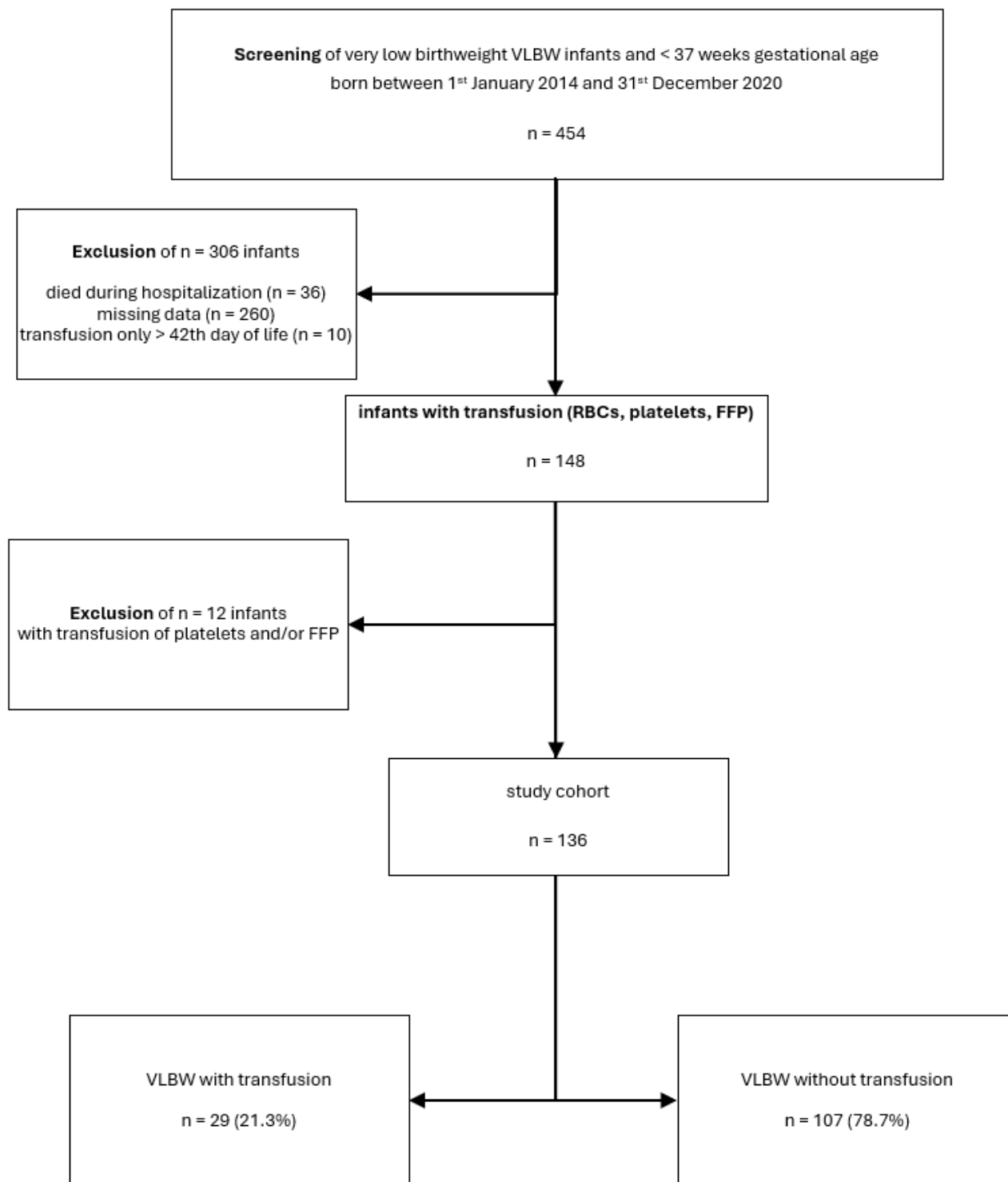


Figure 1

Inclusion and exclusion of infants for current analysis. Abbreviations: RBC red blood cells, FFP fresh frozen plasma

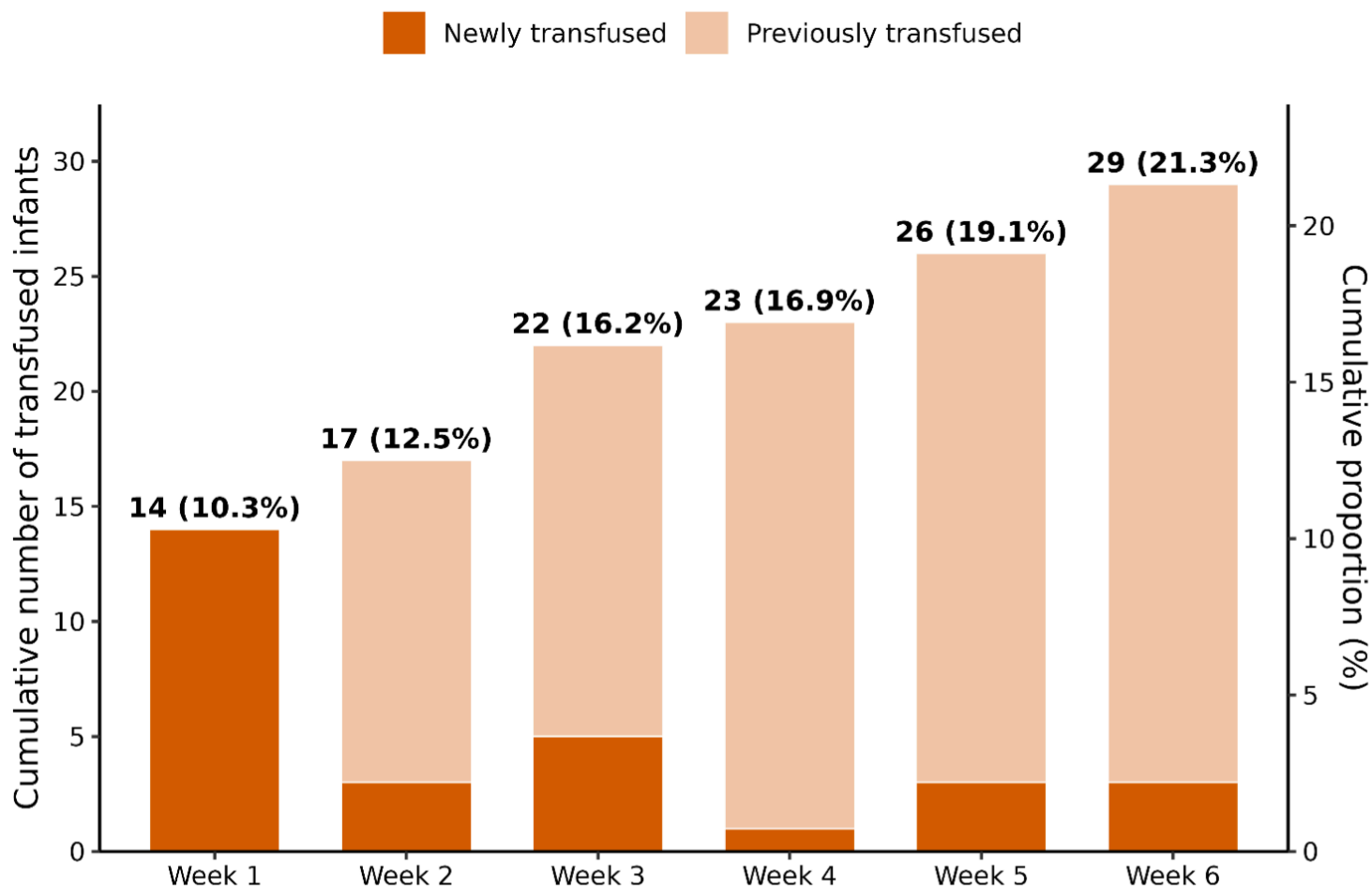


Figure 2

Cumulative proportion of VLBW infants ever transfused with RBCs across the first six postnatal weeks (analysis cohort, N = 136). Bars show the cumulative number of transfused infants per week; the darker (vermillion) segment indicates infants newly transfused within that week, the lighter segment those transfused in a preceding week. Labels above each bar give the cumulative count and the corresponding percentage of the cohort. The right axis shows the cumulative proportion (%).

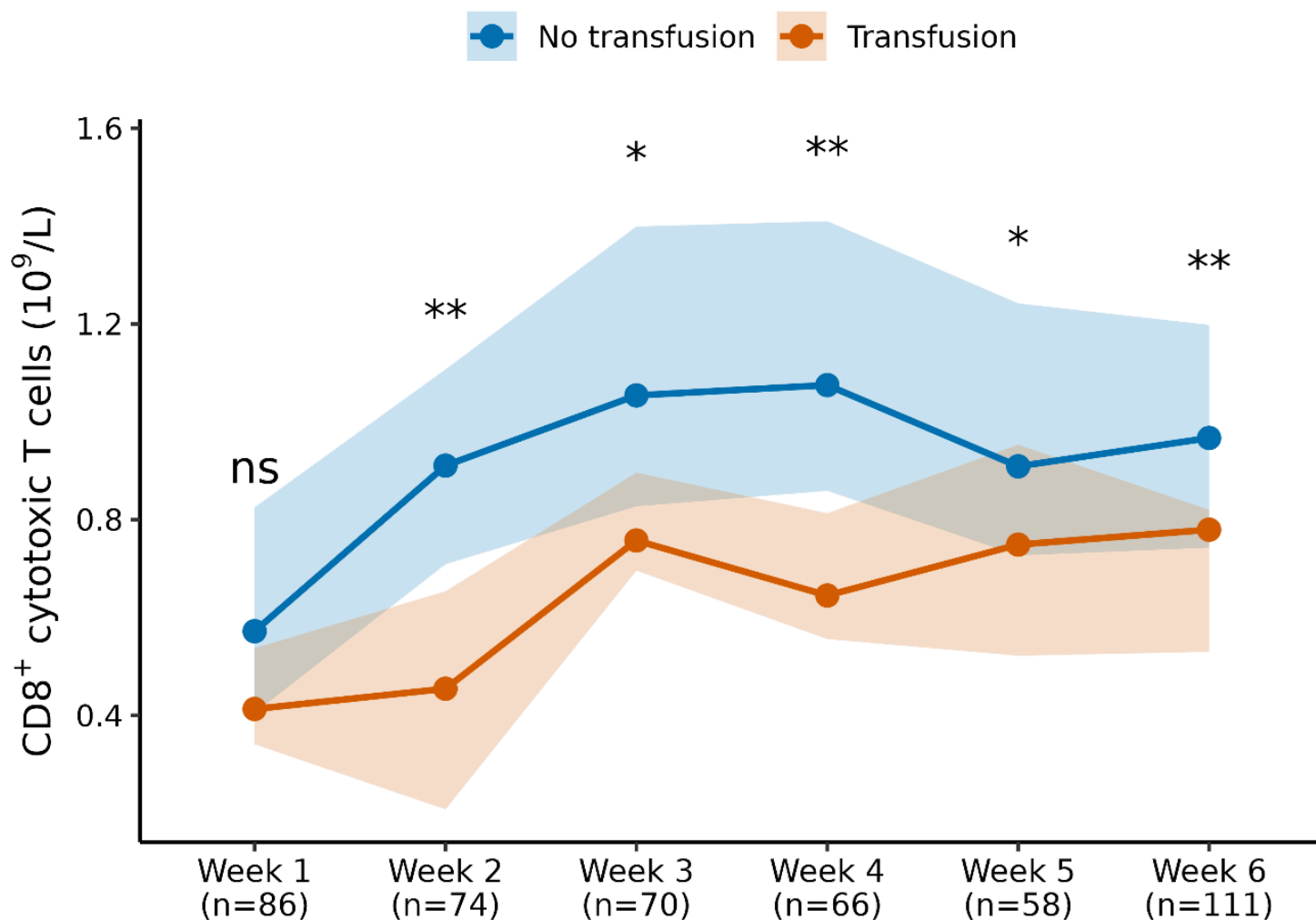


Figure 3

Longitudinal trajectory of CD8⁺ T cell counts over the first 6 weeks of life according to transfusion exposure. CD8⁺ T cell counts (10⁹/L) are shown for transfused and non-transfused very low birthweight (VLBW) infants across the first 6 postnatal weeks. Solid lines represent median values, and shaded areas indicate interquartile ranges (IQR). Non-transfused infants are shown in blue, and transfused infants in orange. Statistical comparisons between groups at each week were performed using Mann–Whitney U test. Significance is indicated as follows: ns (not significant), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

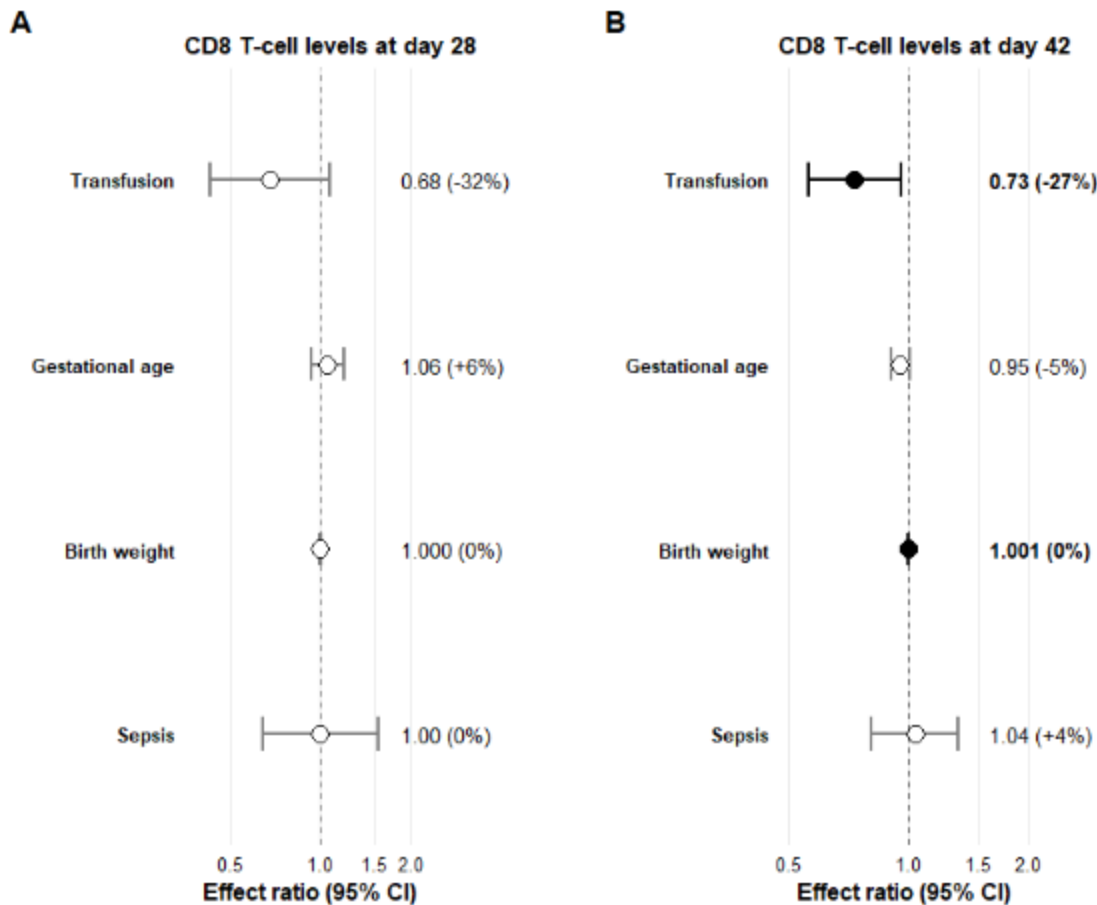


Figure 4

Multivariable associations with CD8+ T cell levels at day of life 28 and day of life 42. Forest plots showing effect ratios with 95% confidence intervals from multivariable linear regression models with log-transformed CD8+ T cell counts as the dependent variable at day 28 (A) and day 42 (B). Covariates included transfusion exposure, gestational age, birth weight, and culture-proven sepsis. Effect estimates are presented as ratios (exponentiated coefficients), with values <1 indicating lower CD8+ T cell levels and values >1 indicating higher levels. The dashed vertical line represents the null value (effect ratio = 1).

Supplementary Files

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