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# Gestational Age-Dependent Changes in Angiogenic and Immunoregulatory Biomarkers Associated with Neonatal Intraventricular Hemorrhage

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## Abstract

Intraventricular hemorrhage (IVH) is a serious complication of the neonatal period, particularly among preterm infants. This study aimed to compare serum vascular endothelial growth factor A (VEGF-A) and transforming growth factor beta 1 (TGF-β1) concentrations in term and preterm newborns according to IVH status and grade. The study included 98 newborns: 26 term newborns without IVH, 22 term newborns with IVH, and 50 preterm newborns with grade I–III IVH. Serum samples were collected during the first week of life, and VEGF-A and TGF-β1 concentrations were measured using commercial enzyme-linked immunosorbent assay kits (Vector-Best, Russia). Mean serum VEGF-A concentrations were 110.38 ± 3.61 pg/mL in controls, 186.82 ± 11.11 pg/mL in term newborns with IVH, and 237.27 ± 22.96, 299.25 ± 24.21, and 342.16 ± 26.14 pg/mL in preterm newborns with grade I, II, and III IVH, respectively. TGF-β1 concentrations were higher in term newborns with IVH than in controls (54.01 ± 2.10 vs. 44.11 ± 2.45 pg/mL), showed no statistically significant difference in the grade I preterm group (44.67 ± 2.08 pg/mL), and were lower in the grade II and III groups (37.24 ± 1.72 and 34.16 ± 1.60 pg/mL, respectively). Thus, VEGF-A and TGF-β1 displayed distinct concentration patterns across the studied groups. Because gestational age differed between groups and adjusted analyses were not reported, these findings should be interpreted as

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exploratory associations. Further studies are needed to determine the clinical significance of these biomarkers.

**Keywords**: Intraventricular hemorrhage; preterm newborns; VEGF-A; TGF-β1; serum biomarkers.

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**Introduction**

Intraventricular hemorrhage (IVH) is a major complication of the neonatal period, particularly in preterm newborns, and is associated with substantial risks of neurological morbidity. The vulnerability of the preterm brain is closely related to developmental immaturity, including the structural fragility of the cerebral vasculature and the incomplete maturation of mechanisms responsible for maintaining stable cerebral blood flow. Fluctuations in cerebral perfusion, systemic instability, hypoxic stress, and inflammatory responses may contribute to vascular injury and hemorrhage. Accordingly, neonatal IVH is considered a multifactorial condition in which gestational maturity and several interacting physiological processes may influence the clinical presentation and severity of injury [7, 11,12,13].

Vascular endothelial growth factor A (VEGF-A) is an important regulator of vascular development and endothelial function. It participates in endothelial-cell proliferation and migration, angiogenesis, and the regulation of vascular permeability. VEGF-A expression and signaling may be influenced by hypoxia, inflammation, and tissue injury. In newborns with IVH, changes in circulating VEGF-A concentrations may therefore reflect systemic vascular or stress-related responses. However, serum VEGF-A levels alone cannot establish the tissue source of the measured protein or determine whether the observed response is protective, maladaptive, or a combination of both. The interpretation of circulating VEGF-A consequently requires consideration of clinical status, developmental maturity, and the timing of sample collection [1,2,3,16].

Transforming growth factor beta 1 (TGF-β1) is a multifunctional cytokine involved in immune regulation, cellular differentiation, extracellular-matrix remodeling, and tissue repair. Its biological effects depend on the cellular environment, developmental stage, and phase of the pathological process. Changes in circulating TGF-β1 concentrations may provide information about systemic immunoregulatory responses; nevertheless, serum measurements do not directly establish cytokine activity within the brain. In neonatal IVH, assessment of TGF-β1 may therefore complement the evaluation of vascular-associated mediators by providing a different perspective on the biological responses accompanying hemorrhagic injury [4,5,6,9].

The simultaneous assessment of VEGF-A and TGF-β1 may be informative because these mediators represent distinct, although potentially interacting, biological processes. VEGF-A is primarily associated with angiogenic signaling and endothelial permeability, whereas TGF-β1 participates in immune regulation and tissue remodeling. Evaluating both markers may help characterize whether their circulating concentration patterns are similar or divergent across newborns with different clinical presentations of IVH. Nevertheless, differences in their serum concentrations do not, by themselves, demonstrate a direct molecular interaction, establish a causal mechanism, or confirm diagnostic or prognostic performance (8,10).

An important challenge in interpreting biomarker patterns in neonatal IVH is the close relationship between gestational age and hemorrhage severity. Preterm newborns with more severe IVH may also differ from those with less severe hemorrhage in developmental maturity and overall clinical condition. Consequently, observed differences in circulating biomarkers may reflect a combination of prematurity, IVH status, hemorrhage grade, and other clinical influences. Comparisons across clearly defined clinical groups can help characterize these differences, but studies incorporating gestational-age-matched controls and appropriate adjustment for potential confounding factors are needed to establish the independent associations of individual biomarkers with IVH (14,15).

Against this background, the present study compared serum VEGF-A and TGF-β1 concentrations in 98 newborns comprising term newborns without IVH, term newborns with IVH, and preterm newborns with grade I, II, or III IVH. The study aimed to characterize the concentration patterns of these two mediators across the clinical groups and to examine their potential relevance as indicators of angiogenesis-associated and immunoregulatory responses in neonatal IVH. Given the observational

comparative design, the findings were interpreted as group-level associations rather than evidence of causality or independently established diagnostic or prognostic value.

## Materials and Methods

### 2.1. Study Design

This study was designed as an observational comparative investigation to evaluate serum concentrations of vascular endothelial growth factor A (VEGF-A) and transforming growth factor beta 1 (TGF-β1) in term and preterm newborns with different clinical presentations of intraventricular hemorrhage (IVH). The analysis compared biomarker concentrations in term newborns without IVH and in newborns with IVH classified according to gestational maturity and the recorded grade of hemorrhage.

The study focused on characterizing differences in circulating angiogenic and immunoregulatory biomarkers between the predefined clinical groups. Because the investigation was observational, the findings were interpreted as associations and were not considered evidence of a causal relationship between biomarker concentrations and IVH development or severity.

### 2.2. Study Population and Group Allocation

A total of 98 newborns were included in the study. Participants were allocated to five groups according to IVH status, gestational maturity, and the recorded grade of hemorrhage.

The control group comprised 26 term newborns without IVH. The remaining 72 newborns had IVH and were divided into four groups: 22 term newborns with IVH, 18 preterm newborns with grade I IVH, 17 preterm newborns with grade II IVH, and 15 preterm newborns with grade III IVH.

The distribution of participants was as follows:

- Group I — Control: 26 term newborns without IVH.
- Group II — Term IVH: 22 term newborns with IVH.
- Group III — Grade I IVH: 18 preterm newborns.
- Group IV — Grade II IVH: 17 preterm newborns.
- Group V — Grade III IVH: 15 preterm newborns.

The mean gestational age was 39.0 ± 0.2 weeks in the term-IVH group, 33.6 ± 0.2 weeks in the grade I group, 30.1 ± 0.3 weeks in the grade II group, and 28.4 ± 0.3 weeks in the grade III group. These differences were considered when interpreting the biomarker results because gestational age and IVH grade were not independent variables in the study population.

### 2.3. Clinical Assessment and IVH Classification

The clinical assessment included examination of the newborns and review of the clinical information used to establish the diagnosis of IVH. Neurosonography was used to identify IVH and determine the hemorrhage grade recorded in the clinical documentation.

Newborns with congenital anomalies and hereditary chromosomal or genetic disorders were excluded, according to the available study information.

The recorded IVH grade was used to classify affected newborns into the respective study groups. The investigation compared serum biomarker concentrations across these groups rather than evaluating the independent diagnostic or prognostic performance of either biomarker.

### 2.4. Blood Sample Collection and Serum Preparation

Blood samples were collected during the first week of life. Serum was prepared in accordance with the laboratory's standard operating procedure (SOP). The same documented laboratory procedure was used for the preparation of samples intended for VEGF-A and TGF-β1 measurements.

The prepared serum samples were used for quantitative determination of the two biomarkers by enzyme-linked immunosorbent assay (ELISA). The pre-analytical procedures were governed by the applicable laboratory SOP and the relevant assay instructions.

Specific pre-analytical parameters, including the blood collection-tube type, clotting time, centrifugation speed and duration, serum storage temperature, storage period, and number of freeze–thaw cycles, should be reported in accordance with the original SOP and laboratory records. These numerical parameters are not stated here because they were not provided in the available study information.

### 2.5. Measurement of Serum VEGF-A and TGF-β1

Serum concentrations of VEGF-A and TGF-β1 were determined using the enzyme-linked immunosorbent assay (ELISA) method. Commercial assay kits manufactured by Vector-Best (Russian

Federation), catalogue No. 677891, were reported as applicable to the measurement of both biomarkers.

The assays were performed according to the manufacturer's instructions and the laboratory SOP. VEGF-A and TGF-β1 concentrations were expressed in picograms per millilitre (pg/mL).

VEGF-A was evaluated as an angiogenesis-associated mediator, whereas TGF-β1 was assessed as an immunoregulatory mediator involved in cellular responses and tissue remodelling. The simultaneous measurement of these biomarkers enabled comparison of their circulating concentration patterns across the five study groups.

The catalogue number and the corresponding assay documentation should be retained in the laboratory records to ensure traceability and reproducibility. Analytical characteristics, including assay sensitivity, detection limits, calibration range, and intra-assay and inter-assay coefficients of variation, should be reported if available in the manufacturer's documentation.

**2.6. Study Variables and Outcome Measures**

The primary outcome measures were serum VEGF-A and TGF-β1 concentrations in the five study groups. The principal comparisons were made between the control group and each IVH group.

Gestational age and available clinical indicators, including Apgar scores, were considered descriptive characteristics relevant to the interpretation of the study findings. The analysis was intended to characterize differences in biomarker concentrations according to IVH status and the clinical groups defined by hemorrhage grade and gestational maturity.

Serum concentrations were interpreted as systemic biomarker measurements. They were not assumed to directly represent biomarker activity within brain tissue or to establish the tissue source of either mediator.

**2.7. Statistical Analysis**

Statistical analysis was performed using Statistica software, version 6.0. Quantitative data were reported as mean ± standard error, consistent with the format used in the study data. Where available, median values and the first and third quartiles [Q1; Q3] were also used to describe the distributions.

Comparisons between independent groups were performed using Student’s t-test when the data met the assumptions required for parametric analysis. A p-value of less than 0.05 was considered statistically significant.

The statistical comparisons were used to assess differences in serum VEGF-A and TGF-β1 concentrations between the control group and the IVH groups. The interpretation of the results took into account the differences in gestational age across the groups.

The specific normality test, assessment of variance homogeneity, sidedness of the statistical tests, and any adjustment for multiple comparisons should be confirmed against the original statistical output before final submission. No unreported statistical procedures were assumed.

**2.8. Ethical Considerations**

The study was conducted in accordance with the ethical approval reported by the research team. Approval was granted by the Ethics Committee of Bukhara State Medical Institute under the Ethics Committee of the Republic of Uzbekistan (approval No. AC123/78).

Written informed consent was obtained from the parents or legal guardians of the participating newborns. The consent documentation was recorded under No. 23, according to the information provided by the study team.

The study involved newborn participants; therefore, the ethical approval and parental or legal-guardian consent documentation should be retained and available for verification if requested by the journal.

**2.9. Methodological Considerations**

The study provides a comparative assessment of serum VEGF-A and TGF-β1 concentrations across newborns with different IVH presentations and a control group without IVH. However, the groups differed in gestational age, and the control group consisted of term newborns. Consequently, the observed biomarker differences may reflect the combined influence of IVH status, hemorrhage grade, prematurity, and other clinical factors.

The findings were therefore interpreted as exploratory group-level associations. The study design does not, by itself, establish the independent diagnostic or prognostic value of VEGF-A or TGF-β1. Further studies with gestational-age-matched control groups, adjustment for relevant clinical confounders, and

longitudinal follow-up would be required to determine the clinical significance of these biomarker patterns.

## Results

### 3.1. Study population, gestational age, and clinical condition

The study included 98 newborns: 26 term newborns without intraventricular hemorrhage (IVH) in the control group and 72 newborns with IVH. The IVH cohort comprised 22 term newborns with IVH, 18 preterm newborns with grade I IVH, 17 with grade II IVH, and 15 with grade III IVH. Thus, the four IVH subgroups differed in both hemorrhage grade and gestational maturity.

Mean gestational age decreased across the preterm IVH subgroups, from 33.6 ± 0.2 weeks in grade I to 30.1 ± 0.3 weeks in grade II and 28.4 ± 0.3 weeks in grade III. The term-IVH group had a mean gestational age of 39.0 ± 0.2 weeks. Apgar scores at 1 and 5 minutes were lowest in the grade III group, indicating a poorer immediate clinical condition in this subgroup compared with the other IVH groups.

**Table 1. Clinical characteristics of the study groups as reported in the source dataset. SE, standard error; IVH, intraventricular hemorrhage. Control-group gestational age and Apgar scores were not provided in the supplied source and are therefore not inferred.**

| Study group | n | Gestational age (weeks), mean ± SE | Apgar at 1 min, mean ± SE | Apgar at 5 min, mean ± SE |
|---|---|---|---|---|
| Control: term, no IVH | 26 | Not reported in source | Not reported in source | Not reported in source |
| Term with IVH | 22 | 39.0 ± 0.2 | 5.4 ± 0.2 | 6.6 ± 0.3 |
| Preterm, IVH grade I | 18 | 33.6 ± 0.2 | 5.5 ± 0.3 | 6.7 ± 0.2 |
| Preterm, IVH grade II | 17 | 30.1 ± 0.3 | 4.2 ± 0.2 | 5.2 ± 0.2 |
| Preterm, IVH grade III | 15 | 28.4 ± 0.3 | 3.1 ± 0.1 | 3.5 ± 0.2 |

### 3.2. Serum VEGF-A concentrations

Mean serum VEGF-A concentration in the control group was 110.38 ± 3.61 pg/mL. The concentration was significantly higher in term newborns with IVH, reaching 186.82 ± 11.11 pg/mL ($p < 0.01$ versus control). Among preterm newborns, mean VEGF-A concentration increased numerically with the reported grade of IVH: 237.27 ± 22.96 pg/mL in grade I, 299.25 ± 24.21 pg/mL in grade II, and 342.16 ± 26.14 pg/mL in grade III; each comparison with the control group was reported as $p < 0.001$.

Calculated from the reported group means, VEGF-A was approximately 1.69-fold higher in term newborns with IVH, 2.15-fold higher in preterm newborns with grade I IVH, 2.71-fold higher in grade II IVH, and 3.10-fold higher in grade III IVH than in controls. These ratios are descriptive comparisons of group means and do not represent adjusted effect estimates. The pattern indicates an increase in

circulating VEGF-A across the IVH groups, while the study design does not establish whether this increase is caused by hemorrhage severity, prematurity, or other clinical factors.

**Table 2. Serum VEGF-A concentrations by study group. Values are transcribed from the supplied manuscript. Q1 and Q3 denote the first and third quartiles.**

| Study group | n | VEGF-A, mean ± SE (pg/mL) | Median [Q1; Q3] (pg/mL) | p vs control |
|---|---|---|---|---|
| Control: term, no IVH | 26 | 110.38 ± 3.61 | 106.75 [97.67; 122.80] | — |
| Term with IVH | 22 | 186.82 ± 11.11 | 203.25 [187.27; 221.34] | <0.01 |
| Preterm, IVH grade I | 18 | 237.27 ± 22.96 | 220.50 [160.55; 270.25] | <0.001 |
| Preterm, IVH grade II | 17 | 299.25 ± 24.21 | 298.66 [201.25; 398.73] | <0.001 |
| Preterm, IVH grade III | 15 | 342.16 ± 26.14 | 321.51 [288.99; 404.52] | <0.001 |

3.3. Serum TGF-β1 concentrations

Mean serum TGF-β1 concentration was 44.11 ± 2.45 pg/mL in the control group. In term newborns with IVH, the mean concentration was higher at 54.01 ± 2.10 pg/mL ($p < 0.01$ versus control). In preterm newborns with grade I IVH, the mean concentration was 44.67 ± 2.08 pg/mL and did not differ significantly from the control value ($p > 0.05$).

In the more severe preterm IVH groups, mean TGF-β1 concentrations were lower than in controls: 37.24 ± 1.72 pg/mL in grade II IVH ($p < 0.05$) and 34.16 ± 1.60 pg/mL in grade III IVH ($p < 0.001$). Based on the reported means, these values were approximately 15.6% and 22.6% lower than the control mean, respectively. The overall pattern differed from that observed for VEGF-A: TGF-β1 was higher in term IVH, similar to control in grade I preterm IVH, and lower in grades II and III. This descriptive pattern should not be interpreted as proof of a specific immunological mechanism.

**Table 3. Serum TGF-β1 concentrations by study group. Values are transcribed from the supplied manuscript. Q1 and Q3 denote the first and third quartiles.**

| Study group | n | TGF-β1, mean ± SE (pg/mL) | Median [Q1; Q3] (pg/mL) | p vs control |
|---|---|---|---|---|
| Control: term, no IVH | 26 | 44.11 ± 2.45 | 41.62 [33.48; 56.59] | — |
| Term with IVH | 22 | 54.01 ± 2.10 | 54.39 [47.67; 60.03] | <0.01 |
| Preterm, IVH grade I | 18 | 44.67 ± 2.08 | 47.00 [39.35; 50.71] | >0.05 |
| Preterm, IVH grade II | 17 | 37.24 ± 1.72 | 36.67 [30.75; 41.46] | <0.05 |
| Preterm, IVH grade III | 15 | 34.16 ± 1.60 | 33.42 [28.40; 37.30] | <0.001 |

### 3.4. Comparative pattern of the two biomarkers

When the two biomarkers were considered together, VEGF-A showed a progressive numerical increase across the IVH groups, reaching its highest mean concentration in grade III IVH. TGF-β1 showed a non-linear pattern: it was elevated in term newborns with IVH, was not significantly different from control in preterm grade I IVH, and was reduced in grades II and III. These contrasting profiles suggest that the circulating angiogenesis-associated and immunoregulatory markers did not change in parallel across the study groups. However, because the groups differed substantially in

gestational age and no adjusted multivariable analysis is reported, the independent relationship of each marker to IVH grade cannot be determined from these data alone.

## Discussion

### 4.1. Principal findings

The present study compared serum concentrations of vascular endothelial growth factor A (VEGF-A) and transforming growth factor beta 1 (TGF-β1) in 98 newborns, including 26 term newborns without intraventricular hemorrhage (IVH) and 72 newborns with IVH. The principal finding was that the two biomarkers followed different patterns across the clinical groups. VEGF-A was higher in every IVH group than in the control group and increased numerically across the groups from term IVH to preterm IVH grade III. In contrast, TGF-β1 was higher in term newborns with IVH, did not differ significantly from control in preterm grade I IVH, and was lower in the grade II and grade III groups. These observations indicate that circulating angiogenesis-associated and immunoregulatory markers did not change in parallel in this cohort.

Interpretation should remain cautious. The groups differed not only by IVH status and reported grade but also by gestational age, with the most severe IVH group having the lowest mean gestational age. Therefore, the data demonstrate differences between the observed groups but do not isolate the independent contribution of hemorrhage severity, prematurity, or other neonatal clinical factors.

### 4.2. VEGF-A elevation across IVH groups

VEGF-A is involved in endothelial-cell survival, proliferation, migration, vascular permeability, and the development and maintenance of blood vessels. Its elevation in all IVH groups is compatible with an activated vascular-response state in newborns affected by IVH. The highest mean concentration was observed in the grade III group (342.16 ± 26.14 pg/mL), compared with 110.38 ± 3.61 pg/mL in term controls. The ratio of these group means was approximately 3.10. This is a descriptive contrast and should not be interpreted as an adjusted effect size or as proof that more severe hemorrhage directly causes a greater VEGF-A response.

Several biological explanations may be plausible, including endothelial stress, vascular remodeling, hypoxia-related signaling, and responses to tissue injury. However, the present study did not measure brain-tissue VEGF-A expression, local vascular permeability, oxygenation, or other mechanistic variables. Serum VEGF-A may reflect systemic processes and cannot by itself identify the brain as the source of the measured protein. Thus, the current findings support an association between IVH-group membership and higher circulating VEGF-A, while the underlying biological pathway remains to be established.

### 4.3. TGF-β1 shows a non-linear group pattern

TGF-β1 is a pleiotropic cytokine involved in immune regulation, cell differentiation, extracellular-matrix turnover, and tissue repair. Its relationship with neonatal brain injury may depend on developmental maturity, the inflammatory environment, timing of sampling, and the balance between injury and repair processes. In the present cohort, the highest mean concentration was observed in term newborns with IVH (54.01 ± 2.10 pg/mL), whereas the grade I preterm group showed no statistically significant difference from controls. Mean concentrations were lower in grade II and grade III IVH, at 37.24 ± 1.72 and 34.16 ± 1.60 pg/mL, respectively.

This non-linear pattern differs from the progressive numerical rise observed for VEGF-A. It may be consistent with differing systemic responses across groups, but the study does not establish whether the lower concentrations in the more severe preterm groups reflect gestational maturity, disease severity, timing of sample collection, altered cytokine regulation, or other factors. In particular, a lower serum concentration cannot be equated directly with reduced TGF-β1 activity in the brain or with a specific failure of tissue repair. Such interpretations would require additional mechanistic and longitudinal evidence.

### 4.4. The combined profile of VEGF-A and TGF-β1

Assessment of the two markers together provides a broader description than either marker alone. VEGF-A increased across the IVH groups, whereas TGF-β1 was elevated in term IVH but decreased in the more severe preterm groups. This divergence suggests that the circulating angiogenesis-associated and immunoregulatory responses may be regulated differently in newborns with IVH. Nevertheless, the study did not perform a formal correlation analysis between VEGF-A and TGF-β1,

and it would be inappropriate to claim a direct relationship, a coordinated molecular pathway, or a predictive biomarker signature from the group-level results alone.

Similarly, the observed concentrations should not be described as a validated diagnostic or prognostic test. No diagnostic cut-off, sensitivity, specificity, area under the receiver-operating-characteristic curve, or association with later neurological outcomes was reported. Further studies would be required to determine whether these markers add clinically useful information beyond gestational age, neurosonography, and established neonatal clinical variables.

**4.5. Gestational age as a major interpretive factor**

Gestational age is a central consideration in this study because neonatal vascular development, immune regulation, and responses to physiological stress change substantially during maturation. The preterm IVH groups had mean gestational ages of 33.6 weeks for grade I, 30.1 weeks for grade II, and 28.4 weeks for grade III. Consequently, the increasing VEGF-A and decreasing TGF-β1 patterns across the preterm groups may reflect both differences in IVH grade and differences in developmental maturity.

The study design does not appear to include gestational-age-matched control groups for each preterm IVH grade, nor does the reported analysis describe multivariable adjustment for gestational age and other potential confounders. The control group consisted of term newborns without IVH, so comparisons with preterm infants may combine the effects of prematurity and IVH. Future investigations should include gestational-age-matched controls where feasible and use appropriately specified multivariable models to assess whether biomarker associations persist after adjustment for gestational age, birth weight, clinical condition, and relevant perinatal factors, provided these variables are systematically collected.

**4.6. Clinical condition and Apgar scores**

The source data also showed lower Apgar scores in the more severe IVH groups. The grade III group had reported mean scores of 3.1 ± 0.1 at 1 minute and 3.5 ± 0.2 at 5 minutes, compared with 5.5 ± 0.3 and 6.7 ± 0.2 in the grade I group. This pattern indicates that the groups differed in immediate clinical condition as well as gestational age and IVH grade. Such differences may be relevant when interpreting systemic biomarker concentrations. However, no individual-level analysis was reported to establish whether VEGF-A or TGF-β1 concentrations were associated with Apgar scores; therefore, a direct relationship between biomarker levels and neonatal condition cannot be concluded.

**4.7. Strengths and limitations**

A strength of the study is the simultaneous assessment of two biologically distinct mediators in a cohort stratified into clinically relevant IVH groups. The inclusion of both mean values and medians with quartiles also provides complementary descriptions of the distributions. The observed contrast between VEGF-A and TGF-β1 identifies a pattern that may be useful for generating hypotheses for future research.

Several limitations should be considered. First, the observational group-comparison design cannot establish causality. Second, gestational age differed substantially between groups, and the control group comprised term newborns, limiting the separation of prematurity-related effects from IVH-related effects. Third, blood was collected during the first week of life, but the exact postnatal sampling time relative to IVH diagnosis and disease evolution was not specified in the source material. Fourth, the supplied manuscript did not report multivariable adjustment, correction for multiple comparisons, correlation analysis, diagnostic-performance measures, or long-term neurological follow-up. Fifth, several numerical discrepancies were present between the source tables and narrative for selected medians, quartiles, and p-values; these must be resolved against the original statistical output before publication. Finally, the ethics committee approval details and parental or legal-guardian consent statement must be confirmed and included in the final manuscript.

**4.8. Implications for future research**

Future studies should prospectively define the timing of blood collection in relation to birth and neurosonographic diagnosis, recruit control groups matched for gestational age, and document relevant clinical covariates. Laboratory methods should report assay catalogue numbers, analytical performance, sample handling, and quality-control procedures. Statistical analysis should prespecify primary comparisons, address multiple testing, and use multivariable models when sample size and data quality permit. Longitudinal follow-up would be necessary to examine whether early VEGF-A and TGF-β1 concentrations are associated with later neurodevelopmental outcomes. Any potential

diagnostic or prognostic use should be assessed in an independent validation cohort rather than inferred from group differences alone.

**4.9. Conclusion of the discussion**

In summary, this study found higher serum VEGF-A concentrations in all IVH groups, with the highest mean in preterm newborns with grade III IVH. TGF-β1 followed a different pattern, being higher in term newborns with IVH, not significantly different from controls in preterm grade I IVH, and lower in grades II and III. These findings describe distinct circulating biomarker patterns across the studied groups. Because gestational age and clinical condition differed between groups and adjusted analyses were not reported, the results should be regarded as exploratory associations requiring confirmation in larger, prospectively designed studies.

## Conclusion

In this cohort of 98 newborns, serum VEGF-A and TGF-β1 showed different patterns in relation to intraventricular hemorrhage (IVH) status, gestational maturity, and the reported severity groups. VEGF-A concentrations were higher in all IVH groups than in the control group and increased progressively from term infants with IVH to preterm infants with grade III IVH. The mean VEGF-A concentration in the grade III group was approximately 3.10 times the control mean. This pattern is consistent with an association between IVH and an intensified angiogenic response, although the observational design does not establish a causal relationship.

TGF-β1 demonstrated a non-linear pattern. Its mean concentration was significantly higher in term newborns with IVH than in controls, did not differ significantly from controls in the preterm grade I group, and was lower in the grade II and grade III groups. These findings suggest that the TGF-β1 profile may vary with clinical group and hemorrhage severity rather than changing uniformly across all newborns with IVH.

Taken together, the findings indicate that VEGF-A and TGF-β1 provide distinct, potentially complementary descriptions of the angiogenic and immunoregulatory milieu in newborns with IVH. However, the present data support group-level associations only; they do not establish either marker, alone or in combination, as a validated diagnostic or prognostic test. Further studies with larger and independently recruited cohorts, clearly documented sampling times, adjustment for gestational age and other clinical confounders, and longitudinal neurodevelopmental outcomes are required to determine the clinical relevance of these biomarker patterns.

## Author Contributions

All Authors contributed equally.

## Conflict of Interest

The authors declared that no conflict of interest

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