

Right-sided venous congestion independently drives amniotic fluid NT-proBNP elevation in TTTS recipient twins

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Abstract

Background

To investigate the differences of amniotic fluid (AF) N-terminal pro-brain natriuretic peptide (NT-pro BNP) levels and cardiac parameters in twin-to-twin transfusion syndrome (TTTS) recipient twins (RTs) across Quintero stages I-IV, and to explore the underlying pathophysiological mechanisms of TTTS.

Methods

The retrospective single-center study enrolled 113 TTTS RTs at Xiangya Hospital between October 2020 and January 2025. All RTs underwent umbilical artery Doppler, echocardiography, CHOP score assessment, and AF NT-pro BNP measurement. Comparisons across stages were performed using Kruskal-Wallis H test. A stepwise multivariate linear regression model was constructed to identify independent predictors of elevated NT-pro BNP. Receiver operating characteristic (ROC) curves were generated to evaluate diagnostic performance.

Results

33 TTTS RTs at Stage I, 39 at Stage II, 34 at Stage III, and 7 at Stage IV were included. Gestational age, DVP, atrial/ventricular transverse diameters were comparable across stages, cardiothoracic area ratio (CTR), CHOP score, and AF NT-pro BNP levels were significantly higher in Stages III and IV compared to Stages I and II ($p < 0.05$), with no significant differences between Stages I and II or between Stages III and IV. After controlling for GA, $\log_{10}$ (NT-pro BNP) showed significant positive correlations with the total CHOP score ($\rho = 0.528$, $p < 0.01$), tricuspid regurgitation ($\rho = 0.452$), and CTR ($\rho = 0.393$). Stepwise multivariate regression confirmed that umbilical vein (UV) pulsation ($\beta = 0.35$, $p < 0.01$), cardiac dilation ($\beta = 0.167$, $p < 0.05$), and umbilical artery pulsatility index ($\beta = 0.094$, $p < 0.05$) were independent positive predictors of $\log_{10}$ (NT-pro BNP). The ROC curve demonstrated an AUC of 0.848 for identifying severe cardiovascular involvement (CHOP > 10). The optimal clinical cut-off was $\log_{10}$ (NT-pro BNP) = 3.75 (approx. 5623pg/ml), yielding a sensitivity of 90.0%. However, NT-pro BNP levels did not differ significantly across definitive pregnancy outcome groups ($p > 0.05$).

Conclusion

AF NT-pro BNP is a highly sensitive biochemical surrogate for quantifying cardiovascular compromise in TTTS RTs. Its elevation is independently driven by right-sided venous congestion, cardiac dilation, and increased placental resistance. It provides excellent diagnostic value for current cardiac strain, but has limited prognostic value for postoperative survival outcomes.

1. Introduction

Twin-to-twin transfusion syndrome (TTTS) is a severe complication affecting approximately 10%-15% of monochorionic diamniotic twin pregnancies^[1]. The underlying pathology involves unbalanced unidirectional blood flow across placental arteriovenous anastomoses, leading to chronic volume depletion in the donor twin and severe volume overload in the recipient twin (RT). Consequently, the RT frequently develops progressive cardiovascular dysfunction, characterized by right ventricular hypertrophy, structural dilation, valvular regurgitation, and ultimately, congestive heart failure or hydrops fetalis^[2]. Currently, the Quintero staging system remains the most widely adopted clinical standard for classifying TTTS severity and guiding treatment decisions^[3]. However, it predominantly relies on ultrasonographic markers, such as amniotic fluid discordance and Doppler abnormalities, which do not directly or comprehensively quantify the extent of myocardial strain^[4]. The cardiovascular score developed by the Children's Hospital of Philadelphia (CHOP) provides a more detailed echocardiographic evaluation^[5]; nevertheless, its calculation is time-consuming, highly operator-dependent, and sometimes challenging to perform in cases of severe polyhydramnios or unfavorable fetal positioning. N-terminal pro-brain natriuretic peptide (NT-proBNP) is a biologically inactive cleavage product co-secreted with BNP primarily by ventricular cardiomyocytes in response to volume expansion and increased transmural wall tension^[6]. Prior investigations have reported elevated AF NT-proBNP levels in TTTS RTs^[7]. However, considering the complex hemodynamic alterations in TTTS and the natural physiological decline of fetal BNP with advancing gestational age (GA), the specific pathophysiological drivers that independently contribute to BNP elevation remain insufficiently elucidated. Therefore, this study aimed to systematically analyze the relationship between AF NT-proBNP, detailed CHOP parameters, and Doppler indices across Quintero stages, utilizing rigorous multivariate adjustment to identify the independent determinants of myocardial strain and evaluate the clinical utility of NT-proBNP.

2. Materials and methods

2.1 Study Subjects

The study was a retrospective analysis carried out at a single center, following the guidelines outlined in the Declaration of Helsinki. Participants were recruited from Xiangya Hospital of Central South University, spanning a period from October 2020 to January 2025. TTTS was identified by the presence of oligohydramnios- polyhydramnios sequence in monochorionic diamniotic twin pregnancies according to the Quintero staging system^[8]. This study excluded cases at Stage V because of the uncertain timing of fetal demise and a lack of sufficient cases during the research period. Following the application of the exclusion criteria, a total of 113 cases from the initial 186 evaluated TTTS RTs were included in the final analysis (Fig. 1). Recorded variables included maternal age, conception methods, and gestational age at amniotic fluid sampling.

2.2 Methods and Indicators

In this research, all enrolled fetuses underwent routine structure examination, echocardiography, umbilical artery (UA) Doppler, CHOP score assessment and amniotic fluid NT-pro BNP measurement.

2.3 Ultrasonographic evaluation

All ultrasound parameters were assessed using a GE Voluson E10 2020 high-resolution color Doppler ultrasound diagnostic system. Cardiac linear parameters and umbilical artery Doppler were obtained according to standard methods^[9]. The cardiothoracic area ratio (CTR) was calculated by employing the ellipse-tracing method to measure both the cardiac and thoracic areas at end-diastole. A ratio between 0.25 and 0.33 was considered normal^[10]. The CHOP score was calculated based on established criteria that evaluate 11 cardiovascular parameters: ventricular hypertrophy, cardiomegaly, left ventricular systolic function, tricuspid valve regurgitation, mitral valve regurgitation, tricuspid valve spectrum, mitral valve spectrum, ductus venosus, pulsatile umbilical vein, right ventricular outflow tract, and pulmonary valve regurgitation. A higher score suggests a more severe heart condition^[11].

2.4 Amniotic fluid NT-pro BNP measurement

For all enrolled fetuses, 3 ml of amniotic fluid was obtained via amniocentesis, before intrauterine therapy or direct puncture during cesarean section. Continuous sampling of the same case is permitted. Amniotic fluid was collected and processed per standard protocols. NT-pro BNP levels were measured via an automated chemiluminescent immunoassay^[7], with validated assay performance characteristics.

2.5 Statistical methods

Data analyses were performed using R software (version 4.5.1) and Python (version 3.10). Continuous variables lacking a normal distribution were expressed as medians with interquartile ranges (IQR). Categorical variables were presented as frequencies and percentages. Non-normally distributed data across multiple groups (e.g., Quintero stages, pregnancy outcomes) were compared using the Kruskal-Wallis H test, followed by post-hoc pairwise Wilcoxon rank-sum tests. Given the skewed distribution of biomarker concentrations, raw AF NT-pro BNP values underwent a log₁₀ transformation [$\log_{10}(\text{NT-pro BNP})$] prior to parametric modeling. To eliminate the recognized confounding effect of fetal development on natriuretic peptide secretion, partial Spearman correlation analysis was employed to assess the associations between $\log_{10}(\text{NT-pro BNP})$ and individual CHOP parameters, strictly incorporating gestational age as the control variable. To determine the independent contributions of respective hemodynamic parameters, a stepwise multivariate linear regression model was constructed. Variables demonstrating clinical relevance and statistical significance in the univariate screening were entered into the initial model. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic accuracy of $\log_{10}(\text{NT-pro BNP})$ in identifying severe cardiovascular involvement, pre-defined as a total CHOP score > 10. The optimal clinical cut-off threshold was calculated using the Youden Index to maximize the sum of sensitivity and specificity. A two-sided p-value < 0.05 was considered indicative of statistical significance.

3. Results

3.1 Comparison of maternal characteristics

The final cohort comprised 113 TTTS RTs. Based on the Quintero staging criteria, they were classified 33 in Stage I, 39 in Stage II, 34 in Stage III, and 7 in Stage IV. No significant differences were found in maternal age, gravidity, parity, gestational age at examination or conception methods among the subgroups. The general maternal characteristics was provided in Table 1.

ART: assistant reproductive technology; GA: gestational age.

Table 1
Maternal demographic and clinical characteristics

variable	total (n = 113)	Stage I (n = 33)	Stage II (n = 39)	Stage III (n = 34)	Stage IV (n = 7)	p value
maternal age (years, mean $\pm$ SD)	30.73 $\pm$ 4.17	32.24 $\pm$ 4.00	30.51 $\pm$ 3.32	29.76 $\pm$ 5.02	29.43 $\pm$ 3.21	0.09
gravidity (n, mean $\pm$ SD)	2.26 $\pm$ 1.44	1.97 $\pm$ 1.45	2.15 $\pm$ 1.23	2.65 $\pm$ 1.61	2.29 $\pm$ 1.50	0.34
parity (n, mean $\pm$ SD)	0.55 $\pm$ 0.65	0.39 $\pm$ 0.66	0.59 $\pm$ 0.59	0.68 $\pm$ 0.73	0.43 $\pm$ 0.54	0.18
ART (n, %)	37 (32.74)	12 (36.36)	14 (35.90)	8 (23.53)	3 (42.86)	0.06
natural conception (n, %)	76 (67.26)	21 (63.64)	25 (64.10)	26 (76.47)	4 (57.14)	0.06
GA (weeks, mean $\pm$ SD)	25.52 $\pm$ 4.74	25.87 $\pm$ 3.07	24.31 $\pm$ 4.96	24.22 $\pm$ 3.93	24.29 $\pm$ 4.66	0.19

3.2 Stage pairwise comparison of cardiac parameters

Pairwise comparison revealed that no significant differences in the left/right atrial/ventricular transverse diameter among TTTS RTs across different Quintero stages. The right ventricular transverse diameter was higher in Stage I than in Stage II ($p = 0.02$), and the left ventricular wall thickness was significantly greater in Stage III than in Stage II ($p = 0.01$). In contrast to the subtle changes in atrial/ventricular transverse diameters and wall thickness, the cardio-thoracic area ratio demonstrated noticeable differences across various Quintero stages, reflecting area data was more sensitive compared to linear measurements. An enlarged CTR became more common with the progression of Quintero stage, cardiac enlargement was present in 51.52% of Stage I cases, rising to 85.71% in Stage IV. The CTR of stages III and IV was significantly higher than that of Stages I and II, but no statistical significance was observed between Stage I and II, or between Stage III and IV (Fig. 2).

LA-TD: left atrial transverse diameter; RA-TD: right atrial transverse diameter; LV-TD: left ventricular transverse diameter; RV-TD: right ventricular transverse diameter; LV-T: left ventricular thickness; RV-T: right ventricular thickness; CTR: cardiothoracic ratio.

Significance levels: * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

3.3 Comparison of AF NT-pro BNP levels

No statistically significant variation was observed in DVP across TTTS groups. AF NT-pro BNP concentrations demonstrated a progressively increasing trend with advancing Quintero stage. Pairwise comparison revealed that AF NT-pro BNP levels were notably elevated in Stages III and IV relative to Stages I/II, however, it was statistically comparable between Stage I and II, and between Stage III and IV. ROC curve analysis was applied to assess the capability of NT-pro BNP as a surrogate diagnostic tool for severe cardiovascular compromise ($\text{CHOP} > 10$). The analysis yielded a high area under the curve (AUC) of 0.848. According to the Youden index, the optimal diagnostic cut-off was established at $\log_{10}(\text{NT-pro BNP}) = 3.75$ (equivalent to 5623pg/ml). At this threshold, the biomarker demonstrated an excellent sensitivity of 90.0% and a specificity of 69.4% (Fig. 3).

Significance levels: ns, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

3.6 Comparison of CHOP score

The mean score was 3.15 ± 3.05 in Stage I, a significant elevation was observed in Stage III, with a mean of 6.32 ± 4.32 and 23.53% (8/34) of cases scoring ≥ 10 ; Stage IV cases exhibited the highest scores, with a mean of 9.71 ± 5.96 and over half (57.14%, 4/7) reaching ≥ 10 . A concordant trend was observed between the CHOP score and the Quintero staging system, however, no statistically discernible difference was observed either between Stage I and II or between Stage III and IV. To delineate the specific morphological and functional drivers of myocardial strain, partial correlation analysis adjusting for GA was performed. The $\log_{10}(\text{NT-pro BNP})$ exhibited a robust positive correlation with the total CHOP score ($p = 0.53$, $p < 0.01$). Analysis of the individual sub-items revealed that markers indicative of right-sided volume overload and venous congestion were most strongly associated with biomarker elevation. Specifically, tricuspid regurgitation ($p = 0.45$, $p < 0.01$), cardiac dilation ($p = 0.39$, $p < 0.01$), abnormal ductus venosus flow (DV, $p = 0.33$, $p < 0.01$), and umbilical vein (UV) pulsation ($p = 0.22$, $p < 0.05$) demonstrated significant positive correlations. A detailed presentation of these results is provided in Fig. 4.

Significance levels: ns, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

3.7 Clinical outcomes and prognostic value of AF NT-pro BNP

Among the 113 TTTS cases, fetoscopic laser surgery was performed in 66 pregnancies (Stage I: 9; Stage II: 27; Stage III: 27; Stage IV: 3). The definitive pregnancy outcomes yielded an overall double survival rate of 73.5% (83/113), a single survival rate of 11.5% (13/113), and a double demise rate of 15.0% (17/113).

When stratified by baseline disease severity, the rates of at least one survivor remained consistently high across Quintero Stages I through IV (84.8%, 79.5%, 82.4%, and 71.4%, respectively), reflecting the efficacy of timely therapeutic intervention. Long-term outcomes, including the longitudinal cardiac and neurological development of the surviving neonates, are currently under ongoing follow-up. Notably, while AF NT-proBNP demonstrated excellent diagnostic accuracy for detecting preoperative cardiac strain, it exhibited limited prognostic value for definitive pregnancy outcomes. Preoperative $\log_{10}$ (NT-proBNP) levels showed no statistically significant differences (Fig. 5) among the double survival, single survivor, and double demise groups (Kruskal-Wallis test, $p > 0.05$). Rather than diminishing the clinical utility of the biomarker, this lack of prognostic correlation underscores the fundamentally reversible nature of TTTS hemodynamics. It implies that successful fetoscopic intervention can effectively unload the fetal heart and alter the disease trajectory, enabling survival even in recipient twins who presented with severe initial biochemical cardiac compromise. Therefore, NT-proBNP is most appropriately utilized as a rigorous indicator of current preoperative myocardial distress rather than an absolute predictor of postoperative fetal demise.

3. Discussion

TTTS is fundamentally a cardiovascular disorder stemming from a volumetric imbalance between the twins^[12]. The recipient twin typically undergoes significant changes in cardiac structure and function^[13], making the assessment of cardiac status crucial for clinical management^[14]. Currently, fetal echocardiography and the CHOP score serve as indispensable non-invasive tools for prenatal cardiac evaluation in TTTS^[15], however, these assessments can be technically challenging or incomplete in up to 16% of cases due to operator dependence or unfavorable fetal positioning^[16]. The extensive application of amniocentesis in TTTS management facilitates easy access to amniotic fluid samples. In our previous research, AF NT-pro BNP has been established as an objective biochemical marker associated with fetal cardiac function^[7]. Given that the Quintero staging system, the primary tool for determining eligibility for fetoscopic surgery, does not incorporate cardiac function^[17], this study aimed to evaluate AF NT-proBNP levels across different Quintero stages, exploring its potential role as an adjunctive assessment tool.

The current study systematically elucidates the mechanisms driving AF NT-pro BNP elevation in TTTS RTs. By applying multivariate statistical models adjusting for gestational age, we observed that NT-pro BNP is not merely a non-specific marker of "cardiac dysfunction" but rather a quantifiable reflection of right-sided volume overload and systemic venous congestion. Our stepwise regression model identified UV pulsation, cardiac dilation, and elevated UA-PI as the principal independent determinants of NT-pro BNP secretion. The predominant role of UV pulsation ($\beta = 0.345$) implies that high-pressure retrograde venous congestion acts as the primary stimulus for BNP release. This congestion is a consequence of right ventricular compliance failure under severe preload^[18]. Concurrently, the retention of cardiac dilation and the elimination of ventricular hypertrophy in the final model corroborate the fundamental physiological premise that BNP is primarily a "volume and wall-tension sensor" rather than a simple

indicator of myocardial thickening. The independent contribution of UA-PI further highlights that placental resistance (increased afterload) exacerbates the myocardial strain initiated by hypervolemia^[19]. These findings align with the hypothesis that right ventricular compromise precedes global left-sided failure in the natural history of TTTS.

In Quintero stages I and II, placental vascular lesions and increased cardiac afterload are still limited, fetal myocardial stretch and strain remain relatively mild, and BNP secretion is accordingly low^[20]. Stage III, defined by absent or reversed UA end-diastolic flow and reversed DV a-wave, marks a marked rise in placental vascular resistance^[21], which combined with hypervolemia stimulates a rapid increase in BNP secretion^[22]. The development of fetal hydrops in stage IV represents definitive evidence of cardiac failure^[23]. At this point, the heart can no longer compensate for the progressively worsening volume and pressure overload. Ventricular filling pressure rises substantially, myocardial damage peaks, and ineffective pumping results in elevated central venous pressure, promoting fluid leakage into the interstitial space (edema). By this stage, cardiac function is severely compromised, and BNP secretion reaches its highest level^[24].

Another key finding of this study is the comparability of AF NT-pro BNP levels, atrial/ventricular transverse diameters, CTR, and CHOP score between Quintero Stages I and II. Similarly, Villa et al. reported no significant difference in CHOP score between the stages^[25]. The clinical utility of the Quintero staging system remains debated in literatures. Some researchers propose merging stages I and II, arguing that the distinction between these two stages is based solely on the presence or absence of a visible bladder in the donor twin, which may not reliably reflect disease progression^[26]. The visualization of the bladder is often dependent on the duration of the examination, and a small, non-dynamic bladder suggests reduced urine output due to inadequate renal perfusion in the donor fetus. In line with this view, Bamberg et al. suggested that differentiating between Stages I and II may be unnecessary, given the absence of significant differences in fetal survival following laser therapy^[27]. Our findings provide quantitative biochemical evidence supporting the conceptual merging of Stages I and II as a more homogeneous clinical entity. Nevertheless, the Quintero staging system remains the standard for fetoscopic surgery, with Stage I managed conservatively and Stage II considered for intervention^[28]. In our cohort, three conservatively managed Stage I cases resulted in double fetal demise and one in single survivor, underscoring the need for improved risk stratification. Clinically, our ROC analysis identified an AF NT-proBNP cutoff of 5623 pg/mL ($\log_{10} = 3.75$), achieving an AUC of 0.848 and 90.0% sensitivity for detecting CHOP scores > 10. In settings where comprehensive echocardiography is technically restricted or fetal positioning precludes detailed assessment, an AF NT-proBNP value exceeding this threshold should prompt clinicians to maintain a heightened suspicion for subclinical, impending cardiac decompensation that may necessitate earlier fetoscopic intervention.

It is noteworthy that CTR, CHOP score and AF NT-pro BNP all demonstrate a clear demarcation between Stages III/IV and Stages I/II. This likely reflects the inclusion of Doppler flow abnormalities as defining criteria for Stage III^[29]. Similar findings have been reported for other cardiac parameters. A study of 290

monochorionic fetuses found that recipient twins in Quintero Stages III + IV demonstrated significantly lower left ventricular A', S', and right ventricular E' values compared to those in Stages I + II or uncomplicated MCDA twins^[29]. Severe atrioventricular valve regurgitation, cardiomegaly, impaired systolic function, and right ventricular outflow tract obstruction also occur with increasing frequency in Stages III and IV^[30]. However, since the Stage IV cohort is the smallest to date, it is not surprising that there is no significant difference in overall survival rates between Stages III and IV. These collective findings reveal a limitation of the Quintero staging system: it cannot fully capture the progressive nature of fetal cardiac dysfunction. As a sensitive, quantifiable, and objective biochemical marker, AF NT-proBNP can be used in conjunction with the Quintero staging system to provide a more comprehensive assessment of fetal cardiac status, offering new pathophysiological insights into TTTS.

Despite the strong correlation with preoperative cardiac strain, AF NT-pro BNP did not exhibit significant prognostic value for final pregnancy outcomes ($p > 0.05$ across survival groups). This negative finding warrants careful interpretation. First, the fact that most of our cases underwent fetoscopic laser ablation, successful prenatal intervention effectively unloads the fetal heart, thereby altering the natural trajectory of the disease^[31]. In this context, a markedly elevated preoperative NT-proBNP level should be interpreted as a sensitive indicator of ongoing myocardial distress rather than a deterministic predictor of inevitable demise. Second, the prognosis of TTTS is influenced by multiple factors^[32], including maternal willingness to undergo surgery, operator technical proficiency, and quality of postnatal neonatal care. These factors collectively contribute to clinical outcomes and should be considered alongside biochemical markers in risk stratification.

Limitations

This study has several limitations. First, the retrospective, single-center design limits generalizability and may introduce selection bias. Second, the unbalanced sample sizes across pregnancy outcome groups may have reduced the statistical power to detect minor prognostic differences. Finally, the absence of longitudinal postoperative AF NT-proBNP data prevents the dynamic evaluation of reverse cardiac remodeling following fetoscopic laser surgery. Future prospective, multicenter studies with serial biochemical sampling and long-term pediatric follow-up are required to validate our proposed diagnostic cut-offs.

4. Conclusion

AF NT-proBNP accurately quantifies the severity of cardiovascular compromise in TTTS recipient twins. The independent pathophysiological drivers of its elevation—venous congestion, cardiac dilation, and placental resistance—highlight the predominance of right-sided overload. Consequently, NT-proBNP should be integrated into clinical practice to identify high-risk cardiac strain and guide surgical timing, rather than to forecast final pregnancy outcomes.

Abbreviations

AF
amniotic fluid
ART
assistant reproductive technology
CHOP
the Children's Hospital of Philadelphia score
CTR
cardiothoracic ratio
DVP
the deepest vertical pocket
GA
gestational age
LA -TD
left atrial transverse diameter
LV -T
left ventricular thickness
LV -TD
left ventricular transverse diameter
NT-pro BNP
N-terminal pro-brain natriuretic peptide
RA -TD
right atrial transverse diameter
RT s
recipient twins
RV -T
right ventricular thickness
RV -TD
right ventricular transverse diameter
TTTS
twin-to-twin transfusion syndrome

Declarations

Ethics approval and consent to participate

The research has been carried out in accordance with the World Medical Association Declaration of Helsinki, this study protocol was reviewed and approved by the Ethics Committee of Xiangya Hospital (protocol code 2025091320, date of approval was Sep. 4th, 2025). Informed consent was obtained from all participants.

Consent for publication

Not Applicable.

Data Availability

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interests.

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Authors' contributions

All authors (WYJ, RJY, YHZ, MP, WJY, GC, RHT, FYT, DWG) contributed to the study conception and design. Material preparation and data analysis were performed by WYJ, MP, DWG, WJY and GC. YJY, YHZ, RHT and FYT contributed to the acquisition of the acquisition and interpretation of data. The first draft of the manuscript was written by WYJ, and all authors (RJY, YHZ, MP, WJY, GC, RHT, FYT, DWG) commented on previous versions of the manuscript, as well as read and approved the final manuscript.

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Figures

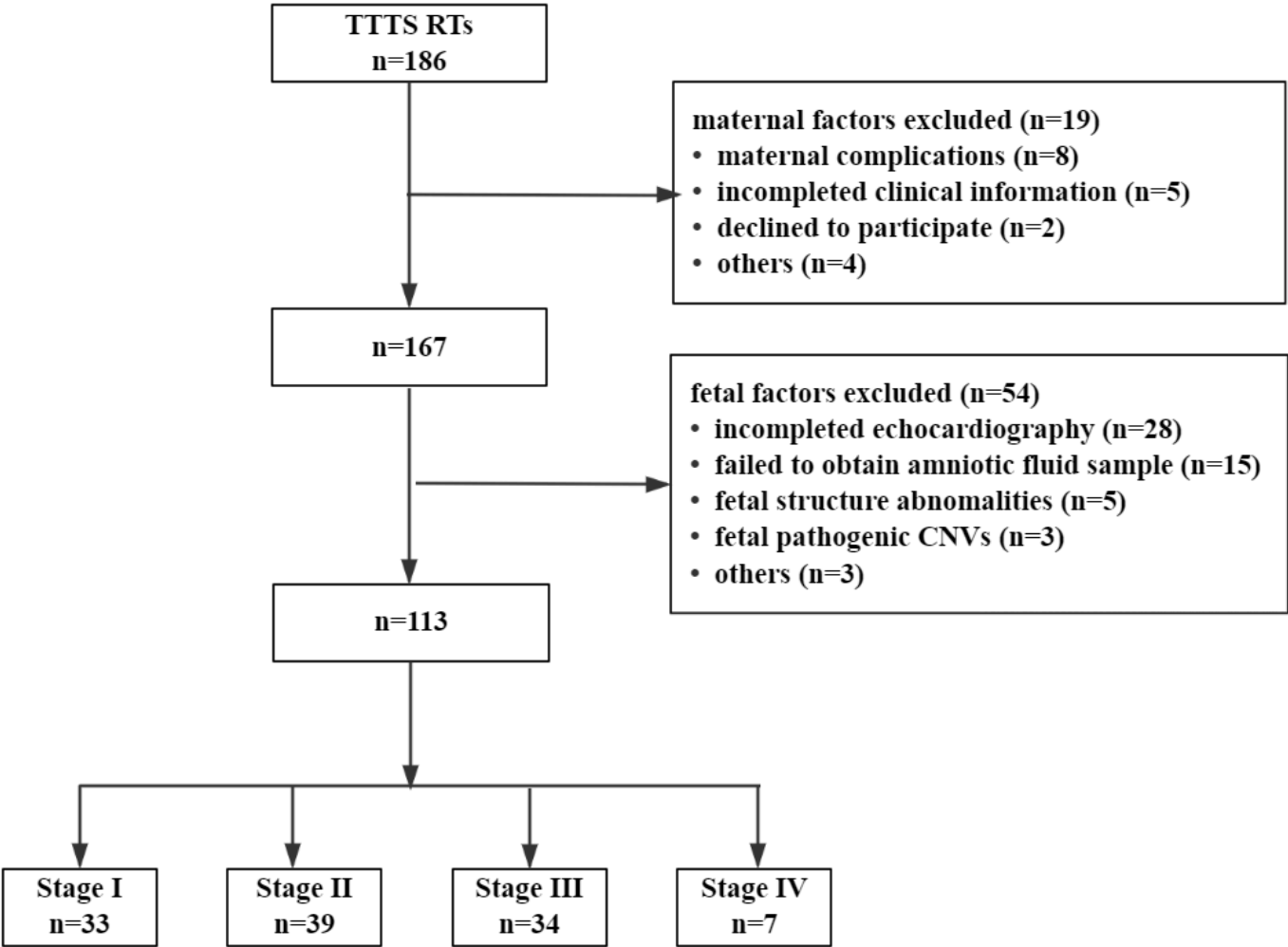


Figure 1

Study flowchart. The final cohort included 113 TTTS recipient twins after applying the exclusion criteria. CNV: copy number variation.

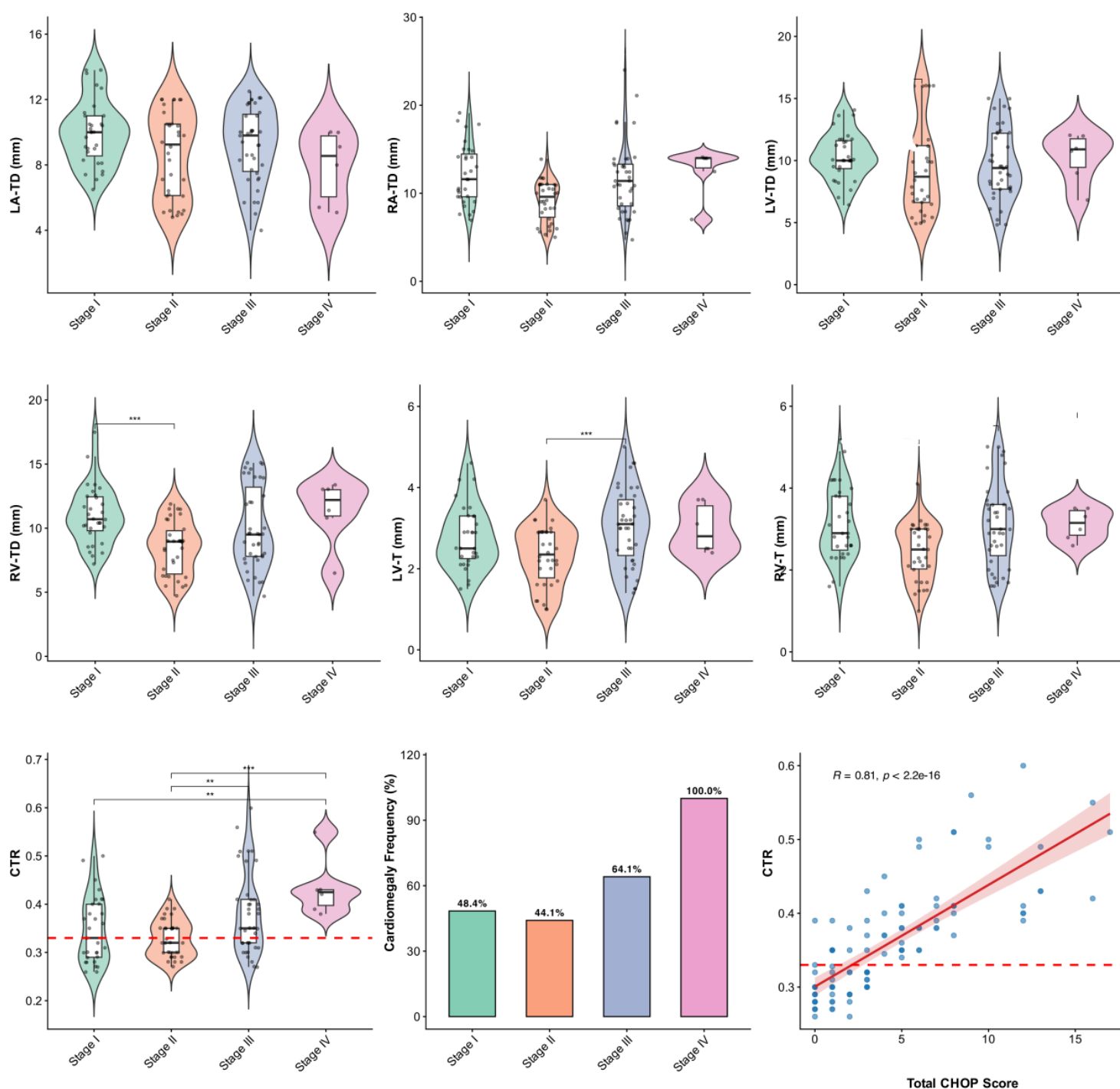


Figure 2

Pairwise comparison of cardiac parameters across Quintero stages. The plot display the distribution of LA-TD, RA-TD, LV-TD, RV-TD, LV-T, RV-T, CTR, frequency of cardiomegaly, correlation of CTR with CHOP score. The red dashed line indicates the upper normal limit.

LA-TD: left atrial transverse diameter; RA-TD: right atrial transverse diameter; LV-TD: left ventricular transverse diameter; RV-TD: right ventricular transverse diameter; LV-T: left ventricular thickness; RV-T: right ventricular thickness; CTR: cardiothoracic ratio.

Significance levels: * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

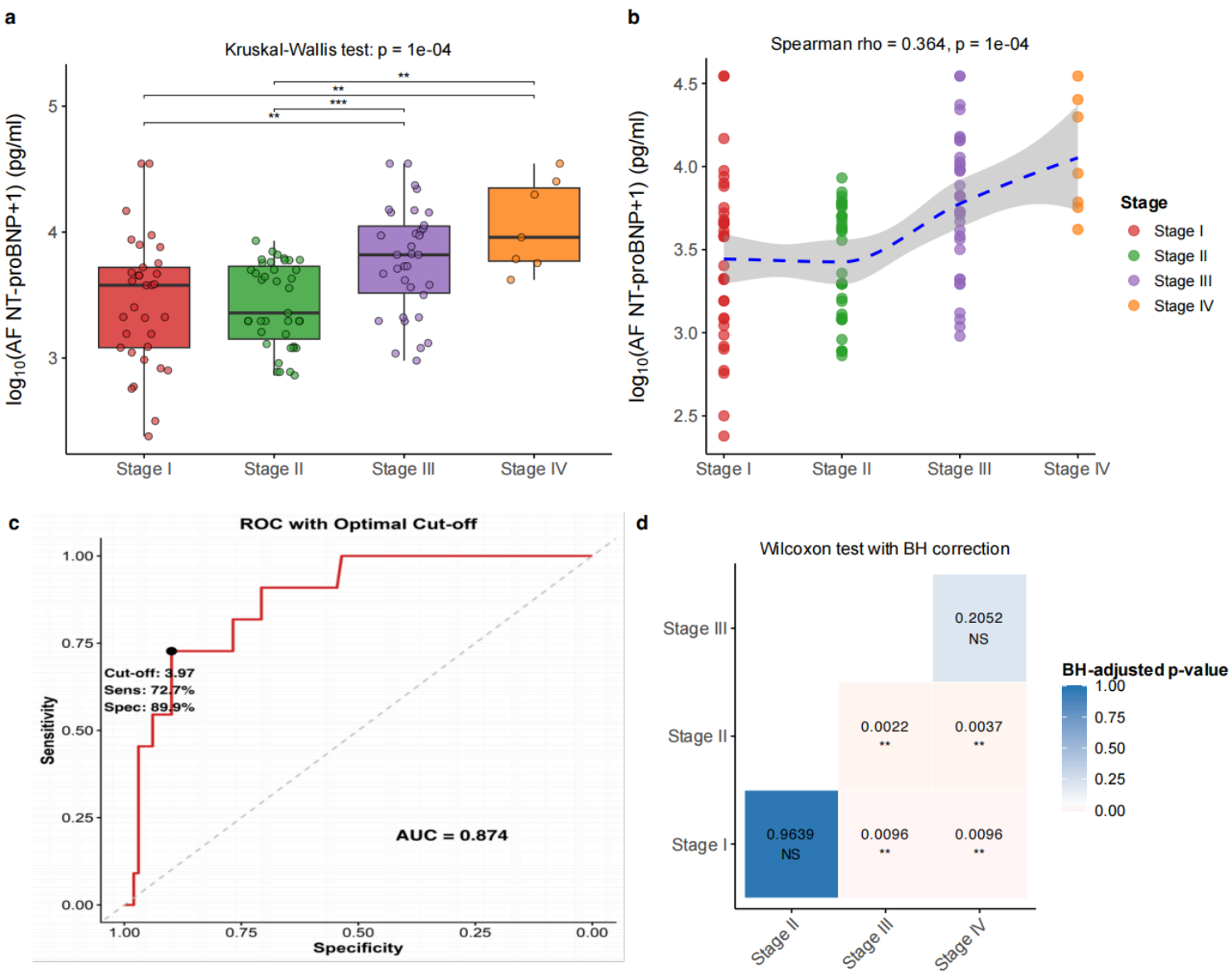


Figure 3

Correlation between AF NT-pro BNP and TTTS Stage. a: $\log_{10}$ (NT-pro BNP) by TTTS Stage. b: Correlation between AF NT-pro BNP and TTTS Stage. c: ROC curve of NT-pro BNP for predicting severe fetal cardiovascular involvement. d: Pairwise Comparisons (BH- adjusted p-values).

Significance levels: ns, $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$.

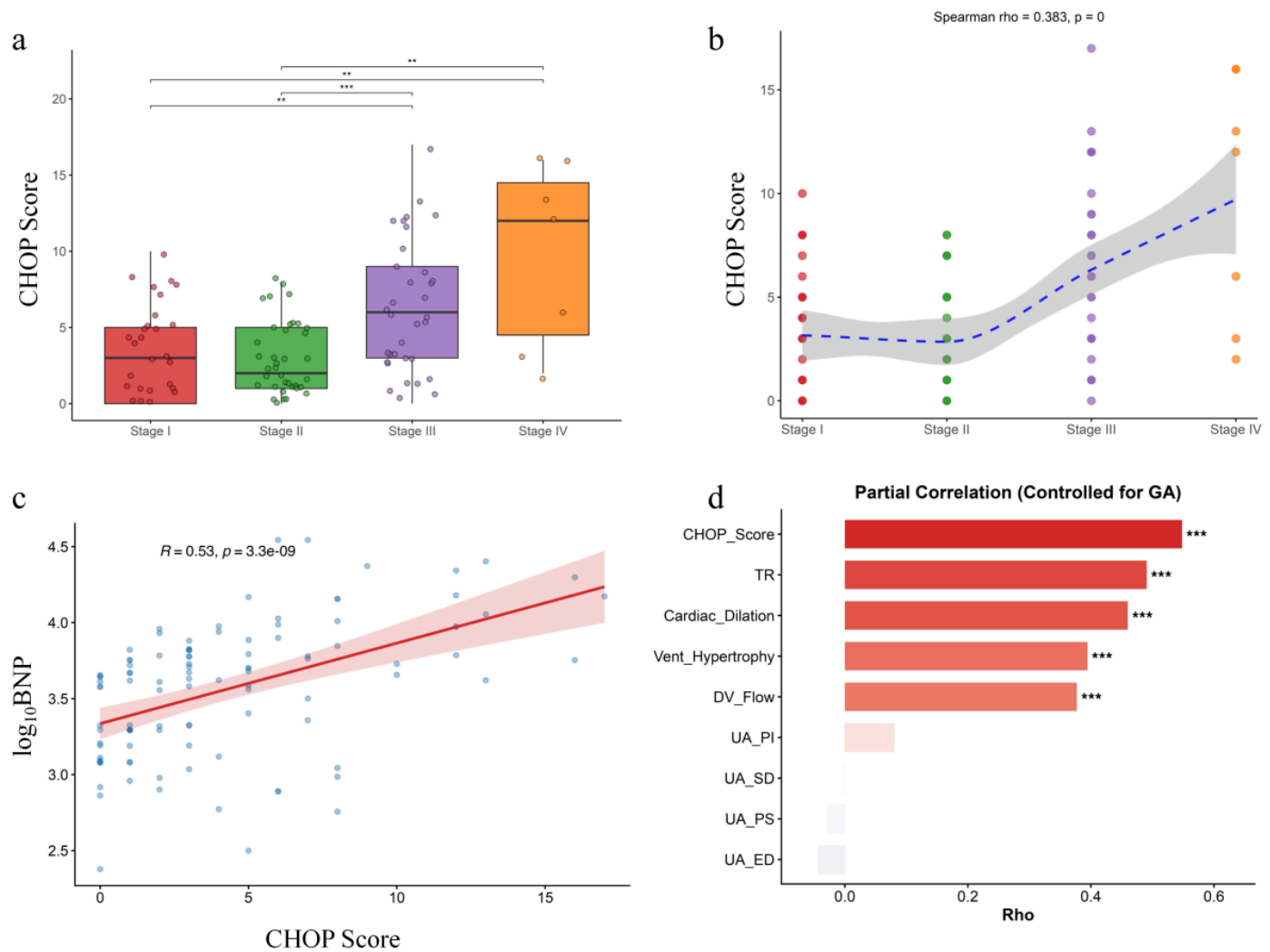


Figure 4

CHOP score across Quintero stages. a: Distribution by stage. b: Positive correlation between CHOP score and Quintero stages. The shaded area represents the 95% CI. c: Scatter plot and linear regression analyzing the relationship between the total CHOP score and log10NT-pro BNP, the shaded area represents the 95% CI. d: Partial Spearman correlation between log10NT-pro BNP and cardiovascular parameters, controlled for gestational age, Bar color intensity reflects correlation strength.

Significance levels: ns, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

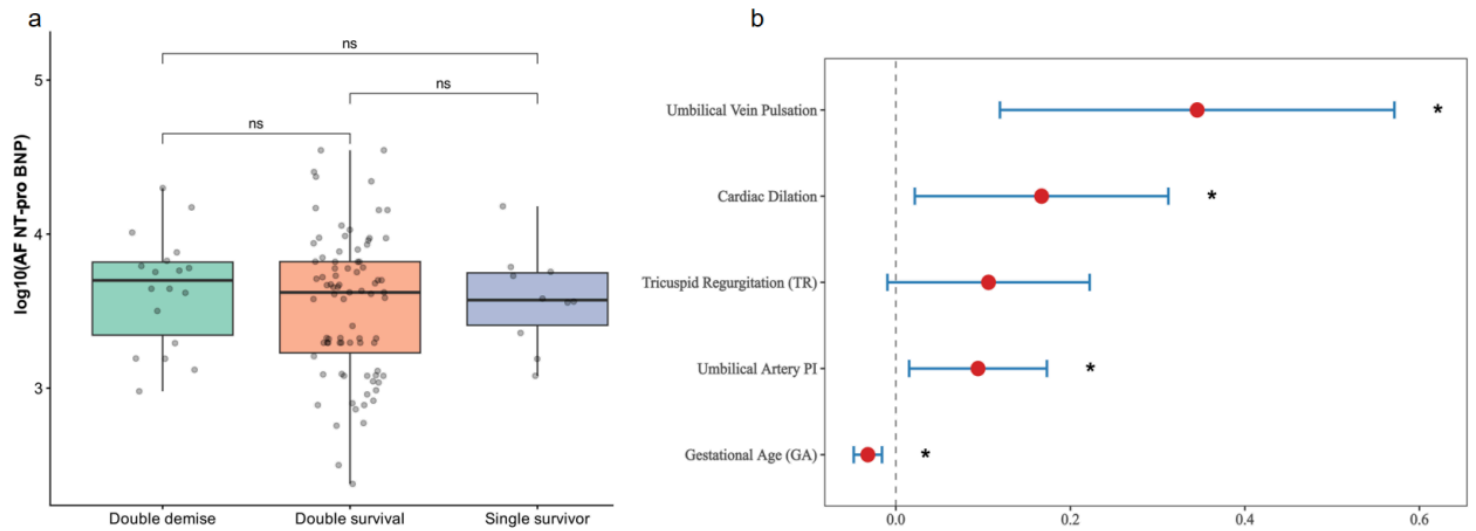


Figure 5

Comparison of preoperative $\log_{10}(\text{AF NT-pro BNP})$ levels among different definitive pregnancy outcome groups. a: The boxplot illustrates the distribution characteristics of the biomarker across the double survival, single survivor, and double demise groups. b: Forest plot of the stepwise multivariate linear regression identifying independent predictors of elevated $\log_{10}(\text{AF NT-pro BNP})$.

ns: $p > 0.05$