

From Static Cutoff to Dynamic Staging: Age-Dependent Diagnostic Thresholds for Serum MMP-7 in Biliary Atresia

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Abstract

Background

Serum matrix metalloproteinase-7 (MMP-7) is a promising biomarker for biliary atresia (BA), but current diagnostic strategies rely on single cutoffs that do not account for disease progression. We aimed to characterize the age-dependent trajectory of MMP-7 in BA and to propose a phase-dependent diagnostic algorithm aligned with the natural history of fibrotic injury.

Methods

This retrospective study consecutively enrolled infants aged ≤ 6 months with pathological jaundice admitted to Guangzhou Women and Children's Medical Center between May 2023 and December 2025. Serum MMP-7 was measured during routine clinical care. A total of 405 eligible infants (193 BA, 212 non-BA) were analyzed. LOESS regression was applied to model the age-related MMP-7 trajectory, and diagnostic cutoffs were derived for distinct biological phases. Robustness was assessed through sensitivity analyses.

Results

BA infants exhibited markedly higher MMP-7 levels than controls (median 76.44 vs. 12.22 ng/mL, $P < 0.001$). The overall cutoff was 30.38 ng/mL (AUC 0.96), but performance varied substantially with age. LOESS regression revealed a three-phase trajectory: an initial phase (≤ 50 days), an escalation phase (51–92 days) characterized by a steep rise, and a plateau phase (> 92 days) with sustained high levels. Based on this staging, phase-specific cutoffs were established: 21.62 ng/mL for infants ≤ 50 days (AUC 0.87, sensitivity 86.4%, specificity 82.9%) and 31.07 ng/mL for those > 50 days (AUC 0.98, sensitivity 95.3%, specificity 96.4%).

Conclusion

The phase-dependent trajectory of serum MMP-7 in BA mirrors progressive fibrotic burden. The proposed three-phase, two-cutoff diagnostic strategy, grounded in the natural history of disease progression, outperforms a single static cutoff and supports a shift toward dynamic, stage-dependent diagnostic frameworks for biliary atresia.

Introduction

Biliary atresia (BA) is a severe hepatobiliary disorder of infancy characterized by progressive inflammation and fibrotic obliteration of intrahepatic and extrahepatic bile ducts, leading to liver fibrosis and cirrhosis [1–4]. Early diagnosis and treatment are critical for restoring bile flow and improving

outcomes [5–9]. Kasai portoenterostomy (HPE) performed within the first 60 days of life is associated with better postoperative outcomes, whereas delayed surgery results in poor prognosis [10, 11]. Conventional diagnostic modalities – including liver function tests, abdominal ultrasound, magnetic resonance cholangiopancreatography, and radionuclide hepatobiliary scintigraphy – have suboptimal accuracy for distinguishing BA from other neonatal cholestatic disorders [1, 2, 5, 6]. Consequently, many infants miss the optimal surgical window.

Multiple studies have reported that serum matrix metalloproteinase-7 (MMP-7) can serve as a reliable biomarker to distinguish BA from other cholestatic diseases [12–21]. Serum MMP7 levels are significantly elevated in patients with BA, and the abnormally elevated MMP7 primarily originates from intrahepatic and extrahepatic biliary epithelial cells, correlating with the progression of liver fibrosis in BA [22, 23]. Reported diagnostic cutoffs for serum MMP-7 vary considerably across studies, ranging from 1.43 ng/mL to 67.40 ng/mL, reflecting substantial inter-center and regional heterogeneity [24–26]. A unified clinical standard is lacking. Moreover, current studies uniformly apply a single diagnostic reference value without considering age or disease progression. BA is a progressive fibrotic disease; as a marker of bile duct injury and fibrotic burden, MMP-7 levels should increase dynamically with disease duration. A single static cutoff may therefore lead to clinical misclassification across different stages.

We hypothesized that serum MMP-7 levels, reflecting the accumulating burden of fibrotic injury, increase with disease duration (approximated by age) in infants with BA. Consequently, a single diagnostic cutoff would be suboptimal, and age-stratified cutoffs would be biologically sound and clinically superior. To test this hypothesis, we performed a detailed age-stratified analysis in a large retrospective cohort, aiming to delineate the dynamic trajectory of serum MMP-7 with age in BA infants, establish a diagnostic threshold framework aligned with disease stage, and compare the performance of this stage-dependent strategy with conventional single-cutoff approaches.

Patients and Methods

1.1 Study Population

This was a retrospective consecutive case–control study using clinically acquired data. We identified all infants aged ≤ 6 months admitted to Guangzhou Women and Children’s Medical Center between May 2023 and December 2025 with a primary diagnosis of pathological jaundice. All patients who met the inclusion criteria and had serum MMP-7 results available from routine clinical testing were consecutively included; no selective sampling was applied.

Inclusion criteria, predefined before data extraction, were as follows. For the BA group, infants had to be aged ≤ 6 months at admission, have serum MMP-7 measured as part of clinical care, and have BA confirmed by surgical exploration and intraoperative cholangiography. For the control group, infants had to be aged ≤ 6 months at admission, have serum MMP-7 measured as part of clinical care, and have a primary admission diagnosis of pathological jaundice with BA excluded either by surgical exploration, by

clinical follow-up showing resolution of cholestasis without surgical intervention, or by genetic testing confirming an inherited metabolic disease that explained cholestasis. Exclusion criteria for both groups were age > 6 months at admission, an inconclusive diagnosis without surgical exploration that could not rule out BA, or absence of MMP-7 measurement during hospitalization.

BA was diagnosed by surgical exploration and intraoperative cholangiography using standard criteria: visualization of only the gallbladder lumen with non-opacification of both intrahepatic ducts and the distal common bile duct; or if the distal common bile duct was patent but the proximal system did not opacify, compression of the common bile duct followed by repeat cholangiography revealing persistent non-opacification of intrahepatic ducts. When the gallbladder was atrophic and fibrotic, precluding cannulation, BA was diagnosed based on concomitant evidence of hepatic cirrhosis.

The study complied with the Declaration of Helsinki. This retrospective analysis was approved by the Research Ethics Committee of Guangzhou Women and Children's Medical Center (approval No. [2026]175A01). The requirement for additional informed consent for this retrospective analysis was waived because the study involved only de-identified data extracted from clinical records, no additional sample collection or patient contact was performed, and all MMP-7 measurements had been obtained as part of routine clinical care with prior parental consent for diagnostic testing.

1.2 Methods

After admission, serum MMP-7 was measured as part of routine clinical care. Venous blood (0.5 mL) was collected, stored at 2–8°C, and sent to KingMed Diagnostics for MMP-7 measurement using magnetic particle chemiluminescence (acridinium ester chemiluminescence analyzer SMART 500s, Chongqing Kosmile Bio-technology Co., Ltd.). The normal reference range was ≤ 18.5 ng/mL, the upper detection limit is 250 ng/mL. No additional sample collection or re-testing was performed for this study.

Demographic data (age, gender), liver function tests (ALT, AST, GGT, TBIL, DBIL, LDH, TBA), and serum MMP-7 results were extracted from the hospital's electronic medical record system and laboratory database. All data were anonymized before analysis. Only patients with documented MMP-7 results were included; there were no missing MMP-7 data in the final cohort. Based on the final discharge diagnosis and follow-up, patients were assigned to the BA or non-BA group. MMP-7 levels were compared between groups, and patients were stratified by age into four predefined groups (≤ 30 days, 31–60 days, 61–90 days, and > 90 days) to evaluate age-specific diagnostic performance.

To minimize bias, the following measures were implemented. All eligible infants during the study period were included consecutively without any selection based on outcome or MMP-7 levels. Laboratory technicians performing MMP-7 assays were blinded to the final diagnosis. The primary data analyst performed the initial receiver operating characteristic (ROC) and regression analyses without knowledge of group assignment; group labels were only revealed after the analysis plan was finalized. Age groups were predefined based on clinical relevance. Final diagnosis (BA or control) was determined by reviewing medical records and operative reports after data extraction, based solely on the predefined gold

standard (intraoperative cholangiography or definitive clinical follow-up), without reference to MMP-7 values. Although batch-to-batch variation cannot be completely excluded, all measurements were performed by the same laboratory using the same instrument and protocol, minimizing variability.

1.3 Statistical Analysis

Statistical analyses were performed using R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were described as counts and percentages; continuous variables as median (interquartile range, IQR) unless otherwise specified. Normality was assessed using the Shapiro–Wilk test. ROC curve analysis was used to determine the optimal cutoff based on the Youden index. The area under the ROC curve (AUC) with 95% confidence interval (CI) was reported. Group comparisons were performed using the Wilcoxon rank-sum test for continuous variables and the χ^2 test or Fisher’s exact test for categorical variables. For multi-group comparisons across the four age strata, the Kruskal–Wallis test was used, followed by Dunn’s test for pairwise comparisons with Bonferroni correction. Effect sizes ($r = Z/\sqrt{N}$) were calculated for significant pairwise differences. Serum MMP-7 had no missing values. For other laboratory variables (e.g., ALT, AST, GGT), missing data were handled by complete case analysis because the proportion of missingness was $< 5\%$.

To characterize the nonlinear trajectory of MMP-7 across age and to delineate distinct phases, we applied piecewise linear regression with an automatic breakpoint detection procedure. An initial simple linear model of MMP-7 on age was fitted, and two change points ($k = 2$) were then estimated using the segmented package (Muggeo) in R, which iteratively minimizes the residual sum of squares. To visually support the identified phases, we additionally fitted a LOESS curve (span = 0.75) and applied phased background shading, providing a smoother depiction of the trend while maintaining the derived change points. The span was selected by minimizing the corrected Akaike Information Criterion (AICC) to balance fit and overfitting. Second-degree polynomials were used for local fitting. The 95% pointwise confidence band was estimated via bootstrap (1,000 replicates). To assess the internal robustness of the proposed age-dependent diagnostic strategy in the absence of an external validation cohort, a 5-fold cross-validation was performed. A pre-specified sensitivity analysis was conducted to evaluate the influence of outliers. Outliers were defined per age stratum as values $< Q1 - 1.5 \times IQR$ or $> Q3 + 1.5 \times IQR$ (Tukey’s rule). After excluding these outliers, all pairwise group comparisons were repeated. The AUC of the combined model was compared with MMP-7 alone using the DeLong test. A two-sided $P < 0.05$ was considered statistically significant.

Results

2.1 Baseline Characteristics

A total of 466 consecutive infants were screened. 61 cases were excluded because of undetermined diagnosis that could not rule out BA (no definitive follow-up or surgical exploration). The final analysis included 405 eligible infants: 193 with BA (105 male, 88 female; median age 61 days, IQR 43–75 days) and 212 controls (157 male, 55 female; median age 61 days, IQR 47–73 days). The control group

consisted of 198 patients with idiopathic infantile cholestasis, 7 with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), 3 with choledochal cyst (CC), 2 with Alagille syndrome, 2 with sodium taurocholate cotransporting polypeptide (NTCP) deficiency (**Figure S1**).

We also analyzed liver function parameters including GGT, ALT, AST, TBIL, DBIL, IBIL, LDH, and TBA. GGT, TBIL and IBIL showed significant differences between the two groups (both $P < 0.05$), with both markers being significantly elevated in the BA group. Age did not differ significantly between groups ($P = 0.684$) (Table 1).

Table 1
Baseline characteristics of BA and control groups

Characteristic	BA (n = 193)	Non-BA (n = 212)	P value
Age, days, median (IQR)	61 (43–75)	61 (47–73)	0.684
Gender, n (%)			
Male	105 (54.4)	157 (74.1)	< 0.001
Female	88 (45.6)	55 (25.9)	
GGT, U/L, median (IQR)	398.0(195.0-716.5)	162.0(90.0-310.0)	<0.001
ALT, U/L, median (IQR)	129.0(87.0-212.0)	137.0(81.0-266.0)	0.523
AST, U/L, median (IQR)	192.0(125.0-275.0)	193.5(112.3-323.3)	0.810
TBIL, $\mu\text{mol/L}$, median (IQR)	147.7(115.8-173.4)	130.5(101.2-166.9)	0.014
DBIL, $\mu\text{mol/L}$, median (IQR)	101.8(79.1-121.8)	90.6(67.1–117.0)	0.053
IBIL, $\mu\text{mol/L}$, median (IQR)	43.7(34.1–57.0)	39.8(29.7–54.5)	0.028
LDH, U/L, median (IQR)	562.0(446.0-682.8)	574.0(460.5–744.0)	0.172
TBA, $\mu\text{mol/L}$, median (IQR)	140.0(107.9-201.7)	140.2(99.2-193.1)	0.324

2.2 Optimal Diagnostic Cutoff for Serum MMP-7

Serum MMP-7 levels were significantly higher in the BA group (median 76.44 ng/mL, IQR: 43.38–135.97) than in the non-BA group (12.22 ng/mL, IQR: 8.87–16.38; $P < 0.001$). The area under the ROC curve was 0.96 (95% CI: 0.93–0.98). When the diagnostic cutoff was set at 30.38 ng/mL, the overall diagnostic performance was optimal, with a sensitivity of 88.1% and a specificity of 94.8%, positive predictive value (PPV) of 93.9% and negative predictive value (NPV) of 89.7% (Fig. 1A).

2.3 Age-Stratified MMP-7 Levels

When we stratified all cases into the four predefined age groups (≤ 30 days, 31–60 days, 61–90 days, and > 90 days), MMP-7 levels were significantly higher in BA than in non-BA infants in every age stratum,

indicating good diagnostic value across all ages (Table 2) .

Table 2
Age-Stratified Serum MMP-7 Levels and Diagnostic Performance in BA vs. Non-BA Infants

BA Group	≤ 30d	31 ~ 60d	61 ~ 90d	>90d
(ng/mL), median (IQR)	(BA = 11,Non-BA = 8)	(BA = 79,Non-BA = 97)	(BA = 85,Non-BA = 80)	(BA = 18,Non-BA = 27)
	40.69	61.10	103.87	174.00
	(28.85,50.89)	(34.79,84.33)	(60.83,173.5)	(85.07,234.72)
Non-BA Group	13.11	12.66	11.94	9.91
(ng/mL), median (IQR)	(8.80,14.72)	(9.29,18.06)	(8.7,16.2)	(8.47,14.75)
P value	0.003	<0.001	<0.001	<0.001
AUC (95% CI)	0.90(0.71-1.00)	0.92(0.88–0.96)	0.99(0.97-1.00)	1.00(1.00–1.00)

When the BA group was analyzed separately, serum MMP-7 levels differed significantly among the four age groups (Kruskal–Wallis $H = 36.98$, $P < 0.001$) (Fig. 2). Further pairwise comparisons showed that all standardized Z values were negative, indicating that levels in older groups were generally higher than those in younger groups. Specifically, the ≤ 30 days group had significantly lower MMP-7 than the 61–90 days group (adjusted $P = 0.018$, effect size $r \approx 0.406$) and the > 90 days group (adjusted $P = 0.002$, $r \approx 0.805$). The 31–60 days group had significantly lower levels than the 61–90 days group (adjusted $P < 0.001$, $r \approx 0.414$) and the > 90 days group (adjusted $P < 0.001$, $r \approx 0.472$). No significant difference was observed between the 61–90 days and > 90 days groups (adjusted $P = 0.827$, $r \approx 0.146$) (Table 3, **Figure S2**).

Table 3
Pairwise Comparisons of Serum MMP-7 Levels Across Four Age Groups in Infants with BA

Comparison Group	Standardized Z value	Adjusted p-value	Sample sizes of the two groups	Effect size r
≤ 30d vs 31-60d	-1.385	0.997	11 + 79 = 90	0.146
≤ 30d vs 61-90d	-3.978	0.018	11 + 85 = 96	0.406
≤ 30d vs > 90d	-4.336	0.002	11 + 18 = 29	0.805
31-60d vs 61-90d	-5.304	0.000	79 + 85 = 164	0.414
31-60d vs > 90d	-4.647	0.000	79 + 18 = 97	0.472
61-90d vs > 90d	-1.484	0.827	85 + 18 = 103	0.146

A pre-specified sensitivity analysis excluding outliers (defined as values $<Q1 - 1.5 \times IQR$ or $>Q3 + 1.5 \times IQR$ within each age stratum, 2 in the ≤ 30 days group, 2 in the 31–60 days group) did not materially alter the pairwise age-group differences in MMP-7 levels, the Kruskal-Wallis test remained significant ($H = 49.1$, $P < 0.001$). Dunn's post-hoc tests with Bonferroni correction confirmed the same pattern of significance as the primary analysis: MMP-7 levels were significantly higher in older age groups, with the only non-significant comparisons being ≤ 30 days vs 31–60 days and 61–90 days vs > 90 days (**Table S1**).

2.4 Relationship Between MMP-7 and Age

To better characterize the temporal dynamics of serum MMP-7 in relation to disease progression, piecewise linear regression with automatic breakpoint detection was applied. The model converged successfully and identified two significant breakpoints at 50 days (95% CI: 31–69 days) and 92 days (95% CI: 75–110 days) (**Figure S3**). Based on these two change points, the entire age range was divided into three distinct phases: Phase I (≤ 50 days), Phase II (50–92 days), and Phase III (> 92 days). A LOESS smooth curve was superimposed on the scatter plot to facilitate visual interpretation, revealing a gradual rise in MMP-7 during Phase I (initial phase), a steep increase during Phase II (escalation phase), and a plateauing trend in Phase III (plateau phase) (Fig. 3).

This trajectory is consistent with our age-stratified analysis, which demonstrated a significant increase in MMP-7 levels between the 31–60 days and 61–90 days groups (adjusted $P < 0.001$), and no further increase beyond 90 days (adjusted $P = 0.827$). Notably, the steep rise between 50 and 92 days encompasses the critical surgical window for Kasai portoenterostomy, reinforcing the biological rationale for age-specific diagnostic cutoffs.

2.5 Age-Specific Optimal Diagnostic Cutoffs for Serum MMP-7

Using a uniform cutoff of 30.38 ng/mL, diagnostic performance varied markedly across age strata, with lower performance in younger infants (**Table S2**), confirming the limitation of a single diagnostic cutoff. Based on the breakpoints determined from piecewise linear regression, pairwise comparison results and the limited sample size in Phase III and the plateau effect, we simplified the age stratification into ≤ 50 days ($n = 140$) and > 50 days ($n = 265$). The optimal cutoffs were 21.62 ng/mL for ≤ 50 days and 31.07 ng/mL for > 50 days (Table 4, Fig. 1B, Fig. 1C).

Table 4
Age-specific serum MMP-7 optimal diagnostic cutoff values

Optimal diagnostic cutoff value(ng/mL)	≤ 50d	>50d
	(BA = 66, non-BA = 74)	(BA = 127, non-BA = 138)
	21.62	31.07
AUC	0.87(0.81–0.93)	0.98(0.97-1.00)
P	<0.001	<0.001
Sensitivity	86.4%	95.3%
Specificity	82.9%	96.4%
PPV	81.43%	96.03%
NPV	87.5%	95.71%
Note: PPV/NPV derived from this cohort, should not be directly extrapolated.		

Gender had no significant effect on serum MMP-7 levels in any age stratum ($P > 0.05$ for all comparisons) (**Figure S4**).

2.6 Internal cross-validation of the age-stratified diagnostic strategy

To assess the robustness of our proposed age-stratified diagnostic strategy in the absence of an external validation cohort, we performed a 5-fold cross-validation using a fixed age cutoff of 50 days (derived from the overall analysis). In each fold, optimal cutoffs for the younger (≤ 50 days) and older (> 50 days) groups were re-calculated from the training set using the Youden index, and then applied to the validation set. Across the five folds, the mean sensitivity was 90.5% (SD 4.7%), specificity 93.0% (SD 1.1%), positive predictive value 91.9% (SD 2.8%), and negative predictive value 91.7% (SD 3.7%). The thresholds for the younger and older groups showed little cross-fold variation (22.88 ± 2.84 ng/mL and 31.10 ± 0.47 ng/mL, respectively), closely approximating the original cutoffs (21.62 ng/mL and 31.07 ng/mL) (**Table S3, Table S4**). These results demonstrate that the dynamic, age-dependent diagnostic model is internally robust and not overfitted to the single-center dataset.

2.7 Combined Diagnostic Performance

We evaluated the added diagnostic value of combining MMP-7 with GGT, the AUC for GGT alone was 0.75 (95% CI: 0.70–0.80). A logistic regression model combining MMP-7 and GGT yielded a predicted probability AUC of 0.96 (95% CI: 0.94–0.98), which was not significantly different from MMP-7 alone (DeLong test, $Z = -0.615$, $P = 0.538$) (Fig. 1D). Thus, adding GGT did not significantly improve the diagnostic performance of MMP-7.

Discussion

In this large retrospective consecutive case–control study with rigorous bias control measures, we demonstrate a strong, positive correlation between serum MMP-7 levels and patient age in BA, providing evidence that MMP-7 serves as a dynamic biomarker reflecting disease progression and fibrosis burden over time.

Our analysis found that MMP-7 levels showed significant statistical differences across all age groups, with BA infants having significantly higher MMP-7 levels than non-BA infants in every stratum. When age was not taken into account, the optimal diagnostic cutoff for serum MMP-7 was 30.38ng/mL, with both sensitivity and specificity exceeding 90%. This result is consistent with multiple previous studies and validates the excellent diagnostic performance of serum MMP-7 for preoperative diagnosis of BA. Consistent with the epidemiological features reported in previous studies [1, 4, 5, 27–29], our cohort had a higher proportion of females in the BA group than in the control group. To exclude gender as a potential confounder for MMP-7 levels, we performed a stratified analysis and found no significant difference in serum MMP-7 levels between males and females across any age stratum (all $P > 0.05$). However, applying the uniform cutoff of 30.38 ng/mL produced markedly variable performance across age strata, with lower performance in younger infants, confirming the limitation of a single diagnostic cutoff.

Further analysis revealed that within the BA subgroup, serum MMP-7 levels also differed significantly among age groups ($H = 36.98$, $P < 0.001$). Pairwise comparisons showed that all standardized Z values were negative, indicating generally higher levels in older groups. Notably, there was almost no difference between the ≤ 30 days and 31–60 days groups (adjusted $P = 0.997$, effect size $r = 0.146$), suggesting that a meaningful rapid increase has not yet occurred in the early phase of disease (approximately the first two months). The difference between ≤ 30 days and > 90 days was statistically significant (adjusted $P = 0.002$) with a large effect size ($r = 0.805$), indicating a fundamental increase from the youngest to the oldest age group. Although the ≤ 30 days group had a small sample size ($n = 11$), the large effect size versus > 90 days suggests robustness. The differences between 31–60 days and 61–90 days ($r = 0.414$) and between 31–60 days and > 90 days ($r = 0.472$) were both significant (adjusted $P < 0.001$), while the difference between 61–90 days and > 90 days was not (adjusted $P = 0.827$, $r = 0.146$). These findings indicate that a clinically meaningful increase occurs between 31–60 days and 61–90 days, with a plateau after 90 days, suggesting that the main rise window centers around 60 days of age.

The nonlinear trajectory of MMP-7 observed in our cohort – a steep rise between 51 and 92 days followed by a plateau – provides a biological foundation for age-stratified cutoffs. Interestingly, the steep rise encompasses the critical surgical window for Kasai portoenterostomy (optimally performed within 60 days) [10, 11]. This pattern aligns with the molecular pathology of BA: older infants have more severe ductal injury and fibrosis, hence higher MMP-7 levels. Early surgery may delay or reverse fibrosis, whereas after 90 days, cirrhosis is often advanced and MMP-7 secretion reaches a steady state, consistent with poorer surgical outcomes.

The independent association of MMP-7 with BA persisted after multivariable adjustment, confirming that its diagnostic value is not explained by chronological age or routine liver biochemistry alone. The poor calibration of the linear logistic model—expected given the pronounced age-dependent nonlinearity – further reinforces the rationale for abandoning a static age-independent cutoff. A dynamic, stage-specific diagnostic strategy is both statistically and biologically appropriate.

The progressive, age-dependent rise of serum MMP-7 observed in our cohort mirrors the natural history of fibrotic injury in BA. This trajectory is plausibly driven by a self-reinforcing molecular axis that links cholangiocyte injury, EMT, and fibrogenesis. Recent mechanistic studies have delineated both upstream regulators and downstream effectors of MMP-7 in this context. At the upstream level, Yang et al. demonstrated that transforming growth factor- β 1 (TGF- β 1) – a master profibrotic cytokine markedly elevated in BA livers – induces overexpression of sulfotransferase family 2b member 1 (SULT2B1) in cholangiocytes. SULT2B1 subsequently activates the Wnt/ β -catenin signaling pathway, leading to transcriptional upregulation of MMP-7 [22]. At the downstream level, Rong et al. showed that MMP-7 directly cleaves E-cadherin on the surface of biliary epithelial cells, releasing soluble E-cadherin and promoting β -catenin nuclear translocation. This process drives epithelial-mesenchymal transition, thereby accelerating liver fibrosis in both in vitro models and a chronic BA mouse model [23]. MMP-7 can further activate latent TGF- β 1 through proteolytic cleavage, establishing a positive-feedback loop (TGF- β 1 $\rightarrow$ SULT2B1 $\rightarrow$ Wnt/ β -catenin $\rightarrow$ MMP-7 $\rightarrow$ TGF- β 1) that perpetuates and amplifies fibrotic signaling [22]. Blockade of MMP-7 with a neutralizing antibody significantly attenuated EMT and liver fibrosis in chronic BA mice, confirming its pathogenic role and therapeutic potential [23].

These mechanistic insights provide a compelling biological basis for the three-phase age-dependent trajectory of MMP-7 delineated in the present study. In the early progressive phase, incipient bile duct injury triggers TGF- β 1 release, initiating SULT2B1/Wnt/ β -catenin/ MMP-7 axis activation and mild MMP-7 elevation. As injury accumulates with age, the TGF- β 1–MMP-7 positive-feedback loop drives an escalation of MMP-7 secretion by cholangiocytes, corresponding to the steep rise between 50 and 92 days. In the plateau phase, advanced cirrhosis with near-maximal fibrotic burden results in sustained high MMP-7 levels.

Based on these findings, we propose a three-phase dynamic trajectory of serum MMP-7 in BA: an initial phase ($\leq$ 50 days), during which bile duct injury and fibrosis are limited and MMP-7 is mildly elevated; an escalation phase (51–92 days), during which fibrosis progresses rapidly and cholangiocytes secrete large amounts of MMP-7, leading to a sharp rise in serum levels; and a plateau phase ($>$ 92 days), in which MMP-7 secretion likely reaches a steady state in advanced fibrosis and no longer increases significantly with age. This dynamic trajectory closely mirrors the natural history of BA and provides clinical evidence that MMP-7 serves as a marker of cumulative fibrosis burden.

If the same diagnostic reference value is used for both younger and older infants, sensitivity will be low in younger infants, increasing the risk of missed diagnosis and delayed surgery. Age-stratified reference values for serum MMP-7 therefore enable more precise diagnosis of BA, mitigating the misclassification

inherent in a single cutoff. This stage-dependent diagnostic paradigm, grounded in the natural history of the disease, represents an upgrade from the traditional static application of biomarkers and aligns with the principles of precision medicine.

In our stratified analysis, the diagnostic performance in the younger groups was lower than that in the older groups. Several outliers were observed in the younger groups, which may have contributed to the slightly lower diagnostic performance. However, we do not know why outliers markedly above the IQR appear in the younger groups, especially individual abnormally high values in the ≤ 30 days group, which seems difficult to explain by the association between age and liver fibrosis severity and requires further studies to explore and validate. To test the stability of our results, we removed those outliers and re-analyzed the data. Pairwise comparisons showed the same pattern of significance as the primary analysis, which confirms the robustness of our findings.

Based on the identified three-phase trajectory, we derived phase-specific diagnostic cutoffs – a three-phase, two-cutoff strategy. Therefore, we suggest that for infants aged ≤ 50 days with MMP-7 levels in a “grey zone” in whom surgery is not immediately planned, serial MMP-7 monitoring may be helpful. If MMP-7 rises markedly within 1–2 weeks, BA is highly suspected and prompt surgical exploration is advised; if levels remain stable or decline, conservative management can be continued. A clinical diagnostic algorithm based on this strategy is presented in **Figure S5**. In infants with a high clinical suspicion of biliary atresia (BA), an atypical or inconclusive MMP-7 result should not delay further diagnostic evaluation when viewed in isolation. Clinical decisions must be guided by the comprehensive integration of all laboratory findings and the patient's overall clinical presentation.

This study has several limitations. First, this single-center retrospective design may introduce selection bias despite consecutive enrollment and blinding. Second, the sample sizes for the ≤ 30 days and > 90 days groups were relatively small, which may introduce statistical imprecision; Third, because this was a retrospective study using clinically acquired data, batch-to-batch variation cannot be completely excluded, although all measurements were performed by the same laboratory using the same instrument. Fourth, the control group consisted of other cholestatic disorders rather than healthy infants, which may introduce spectrum bias. Fifth, we lacked an external validation cohort. However, we performed a rigorous 5-fold cross-validation that confirmed the stability of the age-stratified cutoffs and the consistency of diagnostic performance across different data subsets. Future multicenter prospective studies with standardized MMP-7 assays, correlation with liver biopsy fibrosis scores to establish a “serum fibrosis staging” system, and exploration of MMP-7 dynamics in postoperative follow-up are warranted.

Conclusion

The age-dependent rise of serum MMP-7 in BA underscores its role as a biomarker of progressive fibrotic injury. Implementing the age-stratified diagnostic cutoffs proposed here allows for a more biologically rational and clinically precise application of MMP-7, moving beyond a static diagnostic tool

toward a dynamic indicator of disease stage. Our three-phase, two-cutoff strategy (≤ 50 days: 21.62 ng/mL; >50 days: 31.07 ng/mL) offers superior diagnostic accuracy across different disease stages, reduces the risk of missed and unnecessary diagnoses, and aligns with the critical surgical window for Kasai portoenterostomy. This study supports a paradigm shift from static biomarker interpretation to a dynamic, stage-dependent diagnostic framework for BA.

Abbreviations

biliary atresia (BA)
matrix metalloproteinase-7 (MMP-7)
Kasai portoenterostomy (KPE)
alanine aminotransferase (ALT)
aspartate aminotransferase (AST)
gamma
glutamyl transferase (GGT)
total bilirubin (TBIL)
direct bilirubin (DBIL)
indirect bilirubin (IBIL)
lactate dehydrogenase (LDH)
total bile acids (TBA)
receiver operating characteristic (ROC)
area under the ROC curve (AUC)
confidence interval (CI)
interquartile range (IQR)
akaike information criterion (AICC)
neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD)
choledochal cyst (CC)
sodium taurocholate cotransporting polypeptide (NTCP)
positive predictive value (PPV)
negative predictive value (NPV)
standard deviation (SD)
transforming growth factor- β 1 (TGF- β 1)
sulfotransferase family 2b member 1 (SULT2B1).

Declarations

Ethics approval and consent to participate: This retrospective analysis was approved by the Research Ethics Committee of Guangzhou Women and Children's Medical Center (approval number: [2026]175A01). The requirement for informed consent was waived by the Ethics Committee of Guangzhou Women and Children's Medical Center because of the retrospective nature of this study. This study involved only de-identified data extracted from clinical records; no additional sample collection or

patient contact was performed. All MMP-7 measurements had been obtained as part of routine clinical care with prior parental consent for diagnostic testing.

Consent to publication:

Not applicable.

Conflict of Interest Disclosures:

The authors declare no conflict of interest.

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Author Contribution

Concept and design: ZW, YN. Acquisition, analysis, or interpretation of data: YN, QL, JL, XB, FY, JW, SQ, XC. Drafting of the manuscript: YN, ZW. Critical review of the manuscript for important intellectual content: ZW. Statistical analysis:YN. Obtained funding: ZW, XC. Administrative, technical, or material support: YN, QL, JL, ZW. Supervision: ZW.

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We thank the patients and their guardians for their support. To enhance the clarity of the manuscript, the authors used DeepSeek for language and grammar refinement during its preparation. The authors subsequently reviewed and edited all content and take full responsibility for the final publication.

Data Availability declaration

The datasets generated and/or analyzed during the current study are not publicly available due to institutional ethics restrictions protecting patient privacy, as the data were derived from de-identified clinical records. The data that support the findings of this study are available from the corresponding author upon reasonable request, subject to institutional review board approval.

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Figures

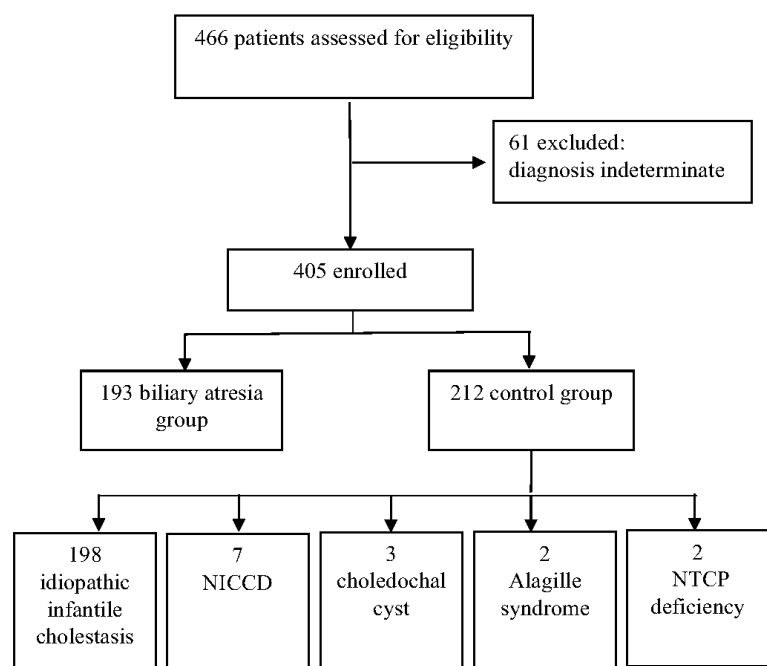


Figure 1

Study Flow Diagram

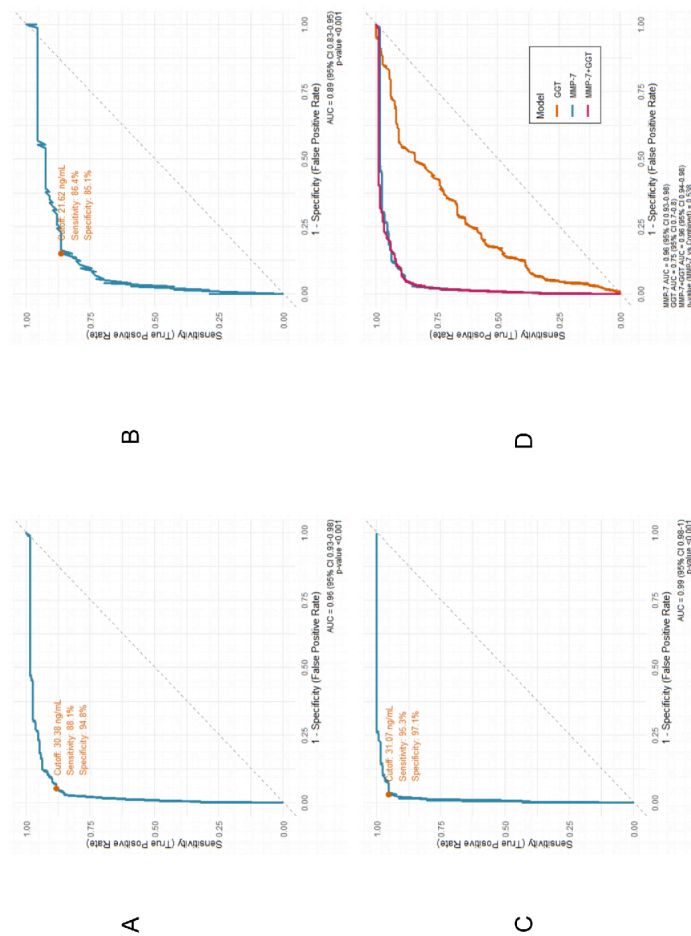


Figure 2

Receiver Operating Characteristic Curves

(A) Overall ROC curve for MMP-7 with optimal cutoff of 30.38 ng/mL (AUC = 0.96).

(B) Age-stratified ROC for infants ≤ 50 days, optimal cutoff = 21.62 ng/mL (AUC = 0.87). (C) Age-stratified ROC for infants > 50 days, optimal cutoff = 31.07 ng/mL (AUC = 0.98). (D) Combined ROC for MMP-7 and GGT (AUC = 0.96), not significantly different from MMP-7 alone (DeLong test, $P = 0.538$).

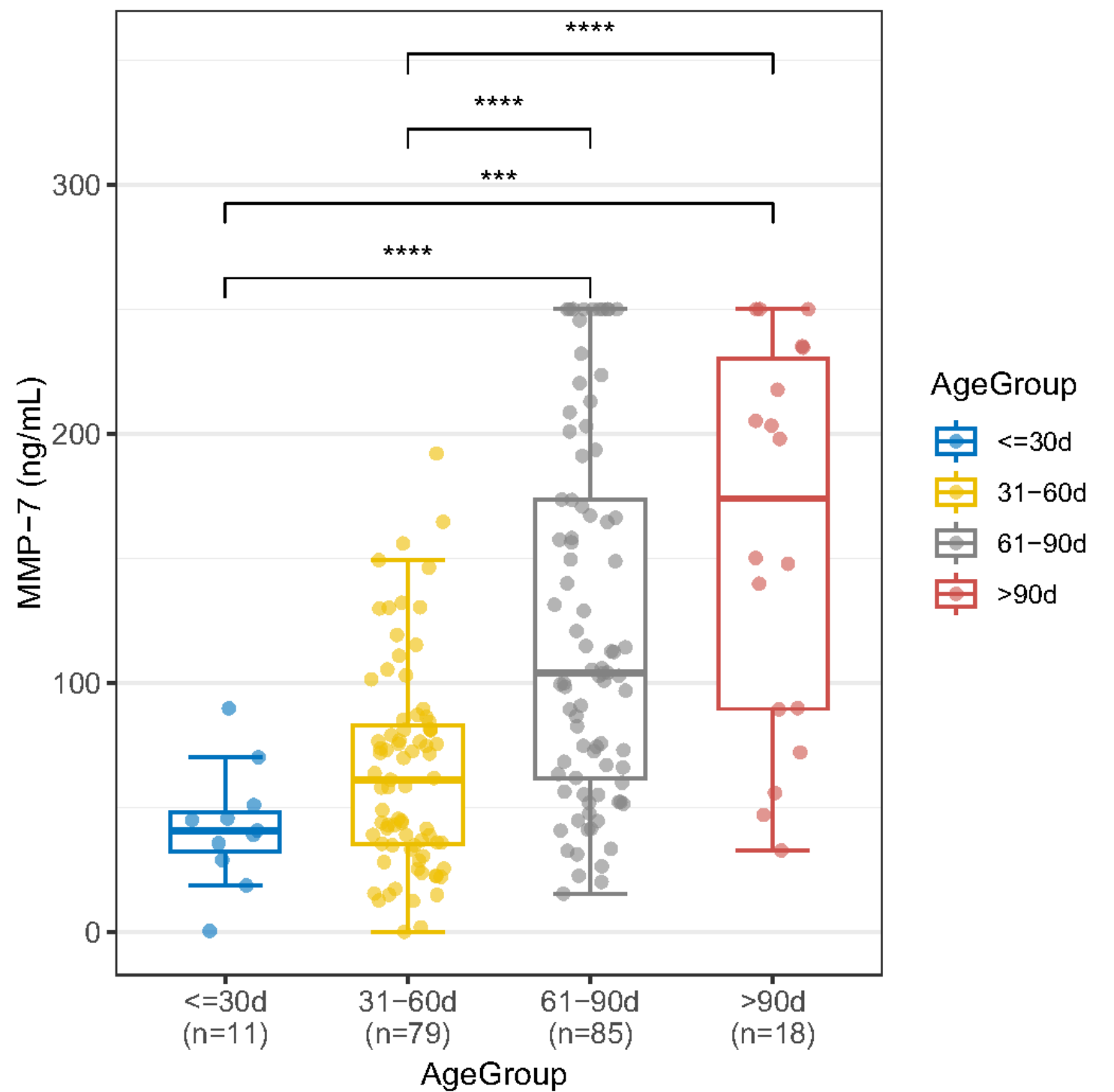


Figure 3

Pairwise Comparisons of Serum MMP-7 Levels Across Four Age Groups in BA Infants

Dunn’s post-hoc test with Bonferroni correction was applied following the Kruskal-Wallis test. Horizontal bars indicate median (IQR). Adjusted P values are shown for each comparison.

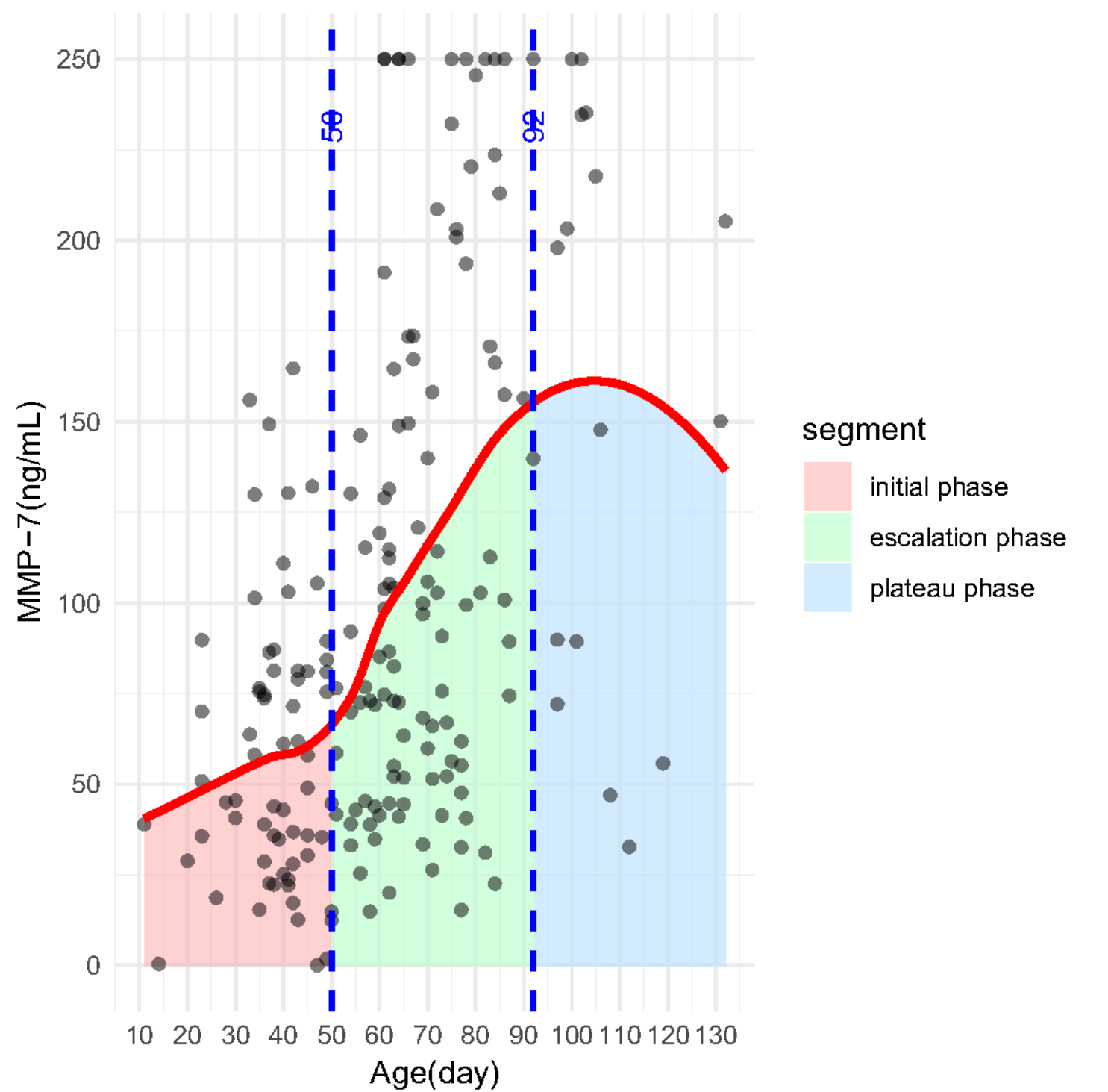


Figure 4

LOESS Smoothed Trajectory of Serum MMP-7 with Age in BA Infants with Phase-Based Stratification

LOESS curve (span = 0.75) overlaid on scatter plot. Phase I (≤ 50 days), Phase II (51–92 days), and Phase III (>92 days) are shaded. The shaded band represents the 95% pointwise confidence interval from 1000

bootstrap replicates.

Supplementary Files

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