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Serological And Cytokine Profiling of Umbilical Cord Blood for Maternal Torch Infections: A Cross-Sectional Study on Neonatal Risk in Yaoundé, Cameroon

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Abstract: Background: Umbilical cord blood (UCB) represents a valuable biological matrix for assessing neonatal exposure to maternal infections and immune status at birth. In resource-limited settings such as Cameroon, its diagnostic potential remains underutilized, particularly for the detection of vertically transmitted infections within the TORCH complex – *Toxoplasma gondii*, Rubella virus *(Rubivirus rubellae)*, *Cytomegalovirus (CMV)/ Human Herpesvirus 5 (HHV-5)*, and *Herpes Simplex Virus* types 1 and 2 (HSV-1/2). Objectives: This study aimed to determine the seroprevalence of TORCH infections in maternal venous blood and UCB, and to compare cytokine profiles in order to characterize neonatal immune activation at birth. Methods: A cross-sectional study was conducted from June to December 2024 across two referral hospitals in Yaoundé, Cameroon. A total of 108 mother-newborn pairs were enrolled consecutively. Paired maternal venous blood and UCB samples were tested using EVANCARE IgM/IgG TORCH rapid diagnostic tests (RDT), and ELISA-based ProcartaPlex™ multiplex cytokine profiling (12 analytes). Results: TORCH seroprevalence in UCB reflected maternal infection status, with high concordance for *Toxoplasma gondii* (90.0%; maternal 77.5% vs. UCB 72.5%) and CMV (70.0%; maternal 55.0% vs. UCB 50.0%). Cytokine profiling revealed that 10 of 12 analytes differed between compartments; IL-6 was significantly higher in maternal plasma (33.94 vs. 25.01 pg/mL; $p < 0.0001$) while IL-2 was significantly elevated in UCB (17.43 vs. 16.54 pg/mL; $p = 0.04$), with IL-4 and IL-5 showing identical values across both compartments. Conclusion: Maternal TORCH infections exert a measurable burden on neonatal infectious and immunological status at birth. UCB represents a non-invasive and ethically advantageous specimen for neonatal assessment. These findings support the integration of TORCH screening and UCB cytokine analysis into routine antenatal care protocols in low-resource settings to reduce neonatal morbidity.

Keywords: umbilical cord blood, TORCH infections, cytokine profiling, neonatal immunity, vertical transmission, Cameroon, sub-Saharan Africa, congenital infections.

INTRODUCTION

Congenital infections remain a leading cause of perinatal morbidity and mortality worldwide, disproportionately affecting populations in sub-Saharan Africa where antenatal screening programmes are limited [1]. The TORCH complex – comprising *oxoplasma gondii*, Rubella virus *(Rubivirus rubellae)*, *Cytomegalovirus (CMV)/ Human Herpesvirus 5 (HHV-5)*, and *Herpes Simplex Virus* types 1 and 2 (HSV-1/2)*Toxoplasma gondii*– encompasses a group of pathogens capable of vertical transmission from mother to foetus, with potentially devastating consequences including miscarriage, intrauterine growth restriction, congenital malformations, and neonatal death [2,3].

In Cameroon, while some data on TORCH seroprevalence in the obstetric population exist [13], no study to date has systematically compared serological profiles between maternal venous blood and UCB while simultaneously assessing cytokine-mediated immune responses. Cytokine profiling offers complementary insight into the functional immunological consequences of infectious exposure: proinflammatory mediators such as interleukin-6 (IL-6) may signal active innate immune engagement, while dysregulation of immunoregulatory cytokines such as IL-2 may reflect immune tolerance or exhaustion in the foetal compartment [4–7].

The present study therefore addresses this gap by investigating the concordance of TORCH seropositivity between maternal and UCB samples, and by characterising the UCB cytokine milieu in exposed versus unexposed neonates. Our findings have direct implications for the design of neonatal screening protocols in low- and middle-income countries (LMICs), where timely identification of congenitally infected infants can alter clinical management and long-term outcomes.

MATERIALS AND METHODS

Study Design, Period, and Setting

A cross-sectional comparative study was conducted from June to December 2024 across the maternity units of two hospitals in Yaoundé, the capital of Cameroon: The Cité Verte District Hospital (HDCV), and the Dominican Saint Martin de Porres Health Centre (CHDSMP). These sites were selected to ensure socioeconomic and geographical diversity within the study population.

Study Population and Eligibility Criteria

The study population consisted of delivering mothers and respective UCB samples, constituting mother-newborn dyads. Recruitment was consecutive and non-probabilistic, enrolling all delivering women who provided written informed consent at the time of admission to the maternity unit.

Inclusion criteria comprised: (i) women aged ≥18 years presenting for term delivery (≥36 weeks of gestational age); (ii) willingness to participate and provide written consent; and (iii) singleton pregnancy. Women with haematological disorders, known autoimmune conditions, or who declined consent were excluded.

Data Collection

Following signed informed consent, a structured data collection form was completed capturing socio-demographic and clinical variables including maternal age, ethnicity, gestational age, mode of delivery, neonatal sex, birth weight, and axillary temperature. Additional clinical information was retrieved from maternal antenatal booklets.

Specimen Collection and Transport

Maternal blood and UCB were collected simultaneously at the time of delivery into K_2EDTA anticoagulant tubes, following guidelines of the Clinical and Laboratory Standards Institute (CLSI) [8]. Tubes were inverted gently 3-5 times to ensure thorough mixing with the anticoagulant. Samples were stored in insulated sampling boxes and transported within two hours to the Molecular and Diagnostic Research Laboratory (MDR Lab) for analysis.

TORCH Serological Testing

TORCH serology was performed using the EVANCARE® IgM/IgG Rapid Diagnostic Test (RDT) kit (Evancare Medical, Nantong, Jiangsu Province, China), a lateral flow immunochromatographic assay that simultaneously detects IgM and IgG antibodies against *Toxoplasma gondii*, Rubella virus, CMV, HSV-1, and HSV-2 [9]. Assays were performed according to manufacturer instructions and validated against internal controls: a test result was considered valid only when the positive control line was clearly visible after the reaction period.

Cytokine Profiling

Plasma cytokine concentrations were measured using the ProcartaPlex™ Human Simplex and Combinable Panel (Thermo Fisher Scientific, Waltham, MA, USA), a multiplexed ELISA-based Luminex platform [10]. Cytokines assessed included IL-1β, IL-2, IL-4, IL-5, IL-6, IL-12p70, IL-13, IFN-γ, GM-CSF, TNF-α, IL-18, and TGF-β1. Assays were performed in duplicate per manufacturer instructions. Plates were read on a Luminex 200™ instrument and data were analysed using ProcartaPlex Analyst software.

Quality Assurance

All laboratory procedures were conducted in compliance with ISO 15189:2022 standards for medical laboratory quality management [11]. Internal quality controls were included with every batch of RDT testing and cytokine assays. Only tests meeting defined acceptance criteria for positive and negative controls were included in the final analysis.

Statistical Analysis

Data were entered and managed in Microsoft Excel 2016. Statistical analyses were performed using R version 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism version 8.4.3. Continuous variables were summarised as means ± standard deviations (SD). Normality of distribution was assessed using the Shapiro-Wilk test.

Chi-square tests were used to compare categorical variables (seropositivity rates) between groups. Analysis of variance (ANOVA) was applied for inter-group comparison of continuous cytokine data. A two-sided p-value <0.05 was considered statistically significant.

Ethical Considerations

This study was nested within an ongoing investigation on immunity in HIV-exposed uninfected neonates (ethical clearance N° BTC-JIRB2022-021). All procedures conformed to the ethical standards of the 1964 Helsinki Declaration and its subsequent revisions. Written informed consent was obtained from all participants prior to enrolment.

RESULTS

Socio-demographic and Clinical Characteristics

A total of 108 mother-newborn pairs were enrolled across the two study sites. The mean maternal age was 27.47 ± 5.82 years, with the majority (57.4%) falling in the 25-34 years age group. Mean maternal weight at delivery was approximately 75 kg, and the mean gestational age at delivery was 39 weeks of amenorrhoea. All neonates were delivered at term, with a mean birth weight of 3,301 g. The majority of deliveries (80.6%) were vaginal. Demographic characteristics are summarised in **Table 1**.

Table 1: Socio-demographic and clinical characteristics of mother-newborn pairs (N = 108)

Characteristic	Category	n (%)	Value (mean ± SD)
Maternal age (years)			27.47 ± 5.82
	18–24	28 (25.9%)	
	25–34	62 (57.4%)	
	≥35	18 (16.7%)	
Maternal weight (kg)			75 [69–85]
Gestational age (weeks)			39,6 [39–40,40]
Mode of delivery	Vaginal	87 (80.6%)	
	Caesarean section	21 (19.4%)	
Neonatal birth weight (g)			3301 ± 412
Neonatal sex	Male	54 (50.0%)	
	Female	54 (50.0%)	
Study site	HDCV	59 (54.6%)	
	CHDSMP	49 (45.4%)	

HDCV: Cité Verte District Hospital; CHDSMP: Saint Martin de Porres Dominican Health Centre. SD: standard deviation.

TORCH Seroprevalence in Maternal Blood and Umbilical Cord Blood

Immunochromatographic TORCH profiling revealed significant overall differences between the maternal and UCB infectious status (Chi-square; *p = 0.009*). The detailed seropositivity rates and concordance patterns are presented in Table 2.

Toxoplasma gondii was the most prevalent pathogen detected in maternal samples (77.5%), with a correspondingly high UCB seropositivity rate of 72.5% and an overall

concordance of 90.0%. CMV seroprevalence was 55.0% in mothers and 50.0% in UCB (concordance: 70.0%). Rubella seropositivity was markedly higher in maternal samples (32.5%) compared to UCB (5.0%), with low concordance (62.5%), likely reflecting differential transplacental transfer kinetics of IgG antibodies for this pathogen.

For HSV-1, an intriguing discordance pattern was observed: UCB seropositivity (30.0%) exceeded maternal seropositivity (17.5%), with 17.5% of dyads showing a maternal-negative/UCB-positive profile – a finding that warrants further investigation and may reflect either cross-reactive antibodies, peripartum exposure, or limitations of the rapid test in the UCB matrix. HSV-2 seropositivity was low in both groups (maternal: 5.0%; UCB: 0.0%), with 95.0% concordance (**Table 2**).

Table 2: TORCH seroprevalence and concordance between maternal venous blood and umbilical cord blood samples (N = 40 pairs presented)

Infection	Maternal Seropositivity n (%)	UCB Seropositivity n (%)	Concordance n (%)	Discordant (M+/UCB−) n (%)	Discordant (M−/UCB+) n (%)
Toxoplasma gondii	31 (77.5%)	29 (72.5%)	36 (90.0%)	3 (7.5%)	1 (2.5%)
Rubella virus	13 (32.5%)	2 (5.0%)	25 (62.5%)	13 (32.5%)	2 (5.0%)
Cytomegalovirus	22 (55.0%)	20 (50.0%)	28 (70.0%)	7 (17.5%)	5 (12.5%)
HSV-1	7 (17.5%)	12 (30.0%)	31 (77.5%)	2 (5.0%)	7 (17.5%)
HSV-2	2 (5.0%)	0 (0.0%)	38 (95.0%)	2 (5.0%)	0 (0.0%)

M+/UCB−: Mother seropositive, UCB seronegative. M−/UCB+: Mother seronegative, UCB seropositive. Overall difference between groups: p = 0.009 (Chi-square).

Cytokine Profiles in Maternal and Umbilical Cord Blood Plasma

Multiplex cytokine profiling of 12 analytes by ProcartaPlex™ ELISA revealed a largely divergent immune milieu between maternal plasma and UCB plasma (Table 3).

Table 3: Comparison of cytokine median concentrations between maternal plasma and umbilical cord blood plasma (N = 108 pairs)

Cytokine	Mother (Median, pg/mL)	Umbilical Cord Blood (Median, pg/mL)	*p-value*
IL-1β	2.49	8.53	0.53
IL-2	**16.54**	**17.43**	**0.04***
IL-4	26.92	26.92	0.72
IL-5	13.82	13.82	0.06
IL-6	**33.94**	**25.01**	**< 0.0001***
IL-12p70	10.81	10.62	0.77
IL-13	27.93	27.93	0.75
IFN-γ	17.07	10.29	0.41
GM-CSF	149.8	150.7	0.09
TNF-α	7.57	7.24	0.54
IL-18	432.1	225.9	0.40
TGF-β1	92.95	97.82	0.18

IL: interleukin; IFN: interferon; GM-CSF: granulocyte-macrophage colony-stimulating factor; TNF: tumour necrosis factor; TGF: transforming growth factor. Values represent median concentrations (pg/mL). Bold text denotes statistically significant differences ($p < 0.05$, ANOVA). *: $p < 0.05$.

Of the 12 cytokines assessed, 10 exhibited differing median concentrations between the two compartments, with statistically significant differences identified for IL-2 (maternal: 16.54 pg/mL; UCB: 17.43 pg/mL; $p = 0.04$) and IL-6 (maternal: 33.94 pg/mL; UCB: 25.01 pg/mL; $p < 0.0001$). Notably, IL-6 concentrations were significantly higher in maternal plasma, while IL-2 was paradoxically elevated in UCB relative to maternal plasma. IL-4 and IL-5 were the only cytokines showing identical median values across both compartments (IL-4: 26.92 pg/mL; IL-5: 13.82 pg/mL), reflecting the immune quiescence of the Th2-skewed foetal environment. The remaining cytokines – including IL-1β, IL-12p70, IL-13, IFN-γ, GM-CSF, TNF-α, IL-18, and TGF-β1 – showed differing medians between groups without reaching statistical significance, indicating biological variability in cytokine transfer or de novo neonatal production (**Table 3**).

DISCUSSION

TORCH Seroprevalence and Concordance Between Maternal and Cord Blood

These findings demonstrate a high seroprevalence of TORCH infections in the obstetric population of Yaoundé, consistent with reports from other sub-Saharan African settings [12,13]. Notably, Sake et al. (2024) previously reported TORCH IgG seroprevalence rates of 34.9% for *Toxoplasma gondii*, 28.9% for Rubella, 28.9% for CMV, and 30.1% for HSV-1 in pregnant women in Yaoundé using the same EVANCARE RDT platform [13]. The markedly higher Toxoplasma seropositivity observed in our cohort (77.5%) may reflect differences in sampling strategy, the restriction to term deliveries, and the well-documented peak in IgG transfer efficiency near delivery.

The 77.5% maternal *Toxoplasma gondii* seropositivity observed in this study aligns with established epidemiological data from Central and West Africa, where environmental exposure to T. gondii oocysts is widespread owing to poor water sanitation and close contact with soil and domestic animals [14].

The high concordance between maternal and UCB seropositivity for T. gondii (90.0%) and CMV (70.0%) provides compelling evidence that UCB accurately mirrors the maternal serological status for these infections. This concordance is biologically plausible: IgG antibodies, the predominant class detectable by serological assays at term, are actively transferred across the placenta via the neonatal Fc receptor (FcRn) pathway, with transfer efficiency increasing throughout the third trimester [15]. Consequently, UCB IgG titres at term are expected to approximate, and in some cases exceed, maternal titres due to active concentration mechanisms.

The markedly lower UCB Rubella seropositivity (5.0% vs. 32.5% maternal) is an intriguing finding that may reflect the relatively lower efficiency of transplacental IgG transfer for Rubella-specific antibodies in this population or may indicate that a proportion of maternal Rubella seropositivity reflects vaccination-induced immunity with waning antibody titres insufficient for efficient placental transfer [16]. This disparity underscores the importance of distinguishing natural infection from vaccine-induced immunity in antenatal screening. The observation that UCB HSV-1 seropositivity (30.0%) exceeded maternal seropositivity (17.5%) is unexpected and raises important methodological and biological questions. Possible explanations include cross-reactive antibody responses to shared HSV epitopes, peripartum exposure during labour, or assay-related issues including

non-specific reactions in the UCB matrix. It is acknowledged that RDT performance may differ between specimen types, and future studies should validate UCB HSV results using gold-standard PCR or Western blot methodologies.

Cytokine Profiling: Distinct Immunological Identity of Umbilical Cord Blood

The divergent cytokine profiles between maternal and UCB plasma constitute perhaps the most compelling evidence in this study that UCB reflects the immunological identity of the neonate rather than a mere extension of the maternal circulation. Among the 12 cytokines assessed, only IL-4 and IL-5 showed identical median concentrations across compartments, while 10 others differed – with 2 reaching statistical significance. This pattern is consistent with the known Th2 polarisation of the feto-placental interface, where IL-4 and IL-5 are actively maintained to suppress inflammatory rejection of the semi-allogeneic foetus [21].

The significantly higher IL-6 in maternal plasma (33.94 pg/mL) compared to UCB (25.01 pg/mL; $p < 0.0001$) is an important finding. While IL-6 is a pleiotropic proinflammatory cytokine elevated in response to infection, its higher maternal concentration may reflect systemic immune activation secondary to the physiological stress of labour and delivery, as well as ongoing TORCH-related immune stimulation in the mother [17]. The relatively lower UCB IL-6 may indicate that the foetal innate immune response is attenuated – a deliberate mechanism to prevent harmful neuroinflammation and tissue damage in the developing neonate [19].

The significantly elevated IL-2 in UCB (17.43 pg/mL) versus maternal plasma (16.54 pg/mL; $p = 0.04$) is an unexpected finding that warrants careful interpretation. IL-2 is the principal T-cell growth factor driving clonal expansion and differentiation of antigen-specific T lymphocytes [20]. Its relative elevation in UCB could reflect nascent antigen-driven T-cell priming in response to in utero TORCH antigen exposure, or may represent a compensatory mechanism to maintain immunological homeostasis in the context of suppressed inflammatory cytokines. Alternatively, this difference, while statistically significant, is of modest magnitude and may not be biologically meaningful in isolation; functional T-cell studies would be required to clarify its significance.

The non-significant differences observed for IL-1ß, IL-12p70, IL-13, IFN-γ, GM-CSF, TNF-α, IL-18, and TGF-ß1 across the two compartments suggest that the broader cytokine milieu is relatively conserved between maternal and cord blood, with only targeted immune mediators showing compartment-specific regulation. TGF-ß1 in particular – a key immunosuppressive cytokine – showed similar medians in both groups (maternal: 92.95 pg/mL; UCB: 97.82 pg/mL; $p = 0.18$), consistent with its constitutive role in maintaining fetoplacental tolerance [22]. Collectively, the cytokine data support the interpretation that UCB represents an immunologically distinct compartment from maternal peripheral blood, with a profile shaped by fetal immune programming, TORCH antigen exposure, and the physiological demands of the intrauterine environment.

Clinical and Public Health Implications

Our data make a compelling case for incorporating UCB-based TORCH screening and cytokine profiling into routine obstetric and neonatal care in Cameroon and comparable settings.

UCB collection is non-invasive, ethically straightforward, and does not subject the neonate to the pain, blood loss, or haemodynamic risks associated with venipuncture in the immediate postnatal period [4,5]. By leveraging this biological resource at the moment of birth – when it is otherwise discarded – clinicians can obtain a rich immunological and infectious disease profile at no additional burden to the mother or newborn.

The high TORCH seroprevalence documented in this cohort highlights a substantial and largely undetected burden of infection that, in many cases, may go unrecognised without routine antenatal screening. In the absence of systematic screening, TORCH-infected neonates risk delayed diagnosis and consequent sequelae including hearing loss, chorioretinitis, neurodevelopmental delay, and immunodeficiency [2,3]. Early identification through UCB profiling creates an opportunity for timely clinical intervention, parental counselling, and targeted follow-up.

Limitations and Strengths

Several limitations of this study should be acknowledged. First, serological diagnosis relied exclusively on rapid immunochromatographic tests, which, while practical in resource-limited settings, carry inherent sensitivity and specificity limitations compared to quantitative ELISA or molecular methods such as Polymerase Chain Reaction. The detection of IgM and IgG antibodies in UCB cannot definitively distinguish active congenital infection from passive transplacental transfer of maternal antibodies; confirmatory nucleic acid amplification testing would be required to resolve this distinction. Notably, IgM detection in UCB has limited interpretive value before 18 months of age due to the predominance of maternally derived antibodies in the neonatal circulation.

Second, the sample size, while adequate for detecting differences in major TORCH pathogens, may have been underpowered for subgroup analyses, particularly for less prevalent infections such as HSV-2.

Notwithstanding these limitations, this study constitutes – to our knowledge – one of the first in Cameroon to provide the integration of serological and functional cytokine data, emphasising a multidimensional assessment of the neonatal immune response that advances beyond simple seroprevalence estimation.

CONCLUSION

This study provides multi-dimensional evidence that umbilical cord blood constitutes a serologically and immunologically distinct specimen from maternal peripheral blood in Yaoundé, Cameroon. Maternal TORCH infections – particularly *Toxoplasma gondii* (77.5%) and CMV (55.0%) – impose a measurable infectious burden on neonates, as evidenced by high UCB seropositivity concordance. The distinct cytokine milieu of UCB, characterised by compartment-specific IL-6 and IL-2 regulation, underscores the immunological individuality of the neonate at birth. These findings support the integration of UCB-based TORCH screening and cytokine profiling into routine antenatal and peripartum care protocols in sub-Saharan Africa.

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COMPETING INTERESTS

The authors declare that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Study conception and design: SATC, JNB, MB, VSMB, PMN, CSS. Data and sample collection: SATC, EMN, AEM, DSM. Statistical analysis: SATC, DSM. Interpretation of results: SATC, MB, PMN, JNB, VSMB, SHR. Manuscript drafting: SATC. Critical revision of the manuscript: JNB, PMN, MB, VSMB, MCN, EMN, AEM, CAMM, CSK, CSS, DSM, SHR. All authors have read and approved the final version of the manuscript.

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