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Short communication

Primary cytomegalovirus (CMV) infection in pregnancy: Diagnostic value of CMV PCR in saliva compared to urine at birth

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ABSTRACT

Background: Due to its ease of collection saliva was recently recommended as the preferred specimen, not only for screening, but also for diagnosis of congenital cytomegalovirus (CMV) infection.
Objective: To compare the diagnostic performance of saliva PCR to urine PCR in infants born to mothers with primary CMV infection during pregnancy.
Study design: We retrospectively analyzed available data of infants tested for CMV DNA in urine and saliva at birth. PCR was performed with RealStar® CMV-PCR Kit 1.0 (altona Diagnostics). Infectious virus was detected in urine by rapid culture.
Results: A total of 133 newborns were eligible for final analysis. Saliva swabs and urine were collected at birth with a time interval of 0–8 days (median 0; IQR 0–1). In 55% of newborns, cord blood was also tested. The overall concordance of saliva and urine PCR was 91% (27 positive, 94 negative). In 12 cases with discordant findings the discrepancy was due to false-negative (n = 2) or false-positive (n = 10) PCR results in saliva. Compared to urine, PCR in saliva showed a positive predictive value of 73%. Viral load in saliva was significantly lower ($p < 0.0001$; Mann-Whitney test) in the 10 false-positive cases than in the 27 cases with concordantly positive results.
Conclusions: Positive CMV PCR results in saliva, especially low positive, have to be confirmed by urine testing. In our opinion detection of CMV by PCR in neonatal urine remains the gold standard for diagnosing congenital CMV infection in infants of mothers with primary infection in pregnancy.

1. Background

Confirmation or exclusion of congenital CMV infection (cCMV) in newborns is important for management and surveillance of infected infants. Detection of CMV in urine by cell culture and later by PCR has been the accepted gold standard for diagnosis of cCMV in newborns. Due to its ease of collection saliva is more suitable for large-scale neonatal cCMV screening, being aware of the risk of false-positive PCR results. Recently saliva was also recommended as the preferred specimen for diagnosis of cCMV after known maternal infection [1], although data on the diagnostic accuracy of CMV PCR in saliva compared to urine are limited [2].

2. Objectives

We compared the diagnostic performance of saliva PCR to urine PCR in infants born to mothers with primary CMV infection during pregnancy.

3. Study design

As a medical laboratory with special focus on diagnosis and management of infections in pregnancy, we receive a considerable number of maternal, fetal and neonatal samples for diagnosis of CMV infection. In 2017 we modified our recommendation for diagnosis of cCMV in the newborn to investigate saliva in addition to urine.

For this retrospective study we searched our laboratory information system for saliva samples of newborn infants obtained within the first two weeks of life and tested for CMV DNA. We included only infants of mothers with serological evidence of primary CMV infection in pregnancy. Furthermore all cases without a neonatal urine sample (in addition to saliva) and those who underwent antenatal antiviral therapy were excluded. Data on laboratory test results and, if available, additional information (e.g. timing of sampling, mode of delivery, antenatal treatment) were retrospectively extracted and anonymized before final analysis. In daily practice, we advise to collect the infant's saliva by swabbing inside both cheeks and to send the swab placed in virus

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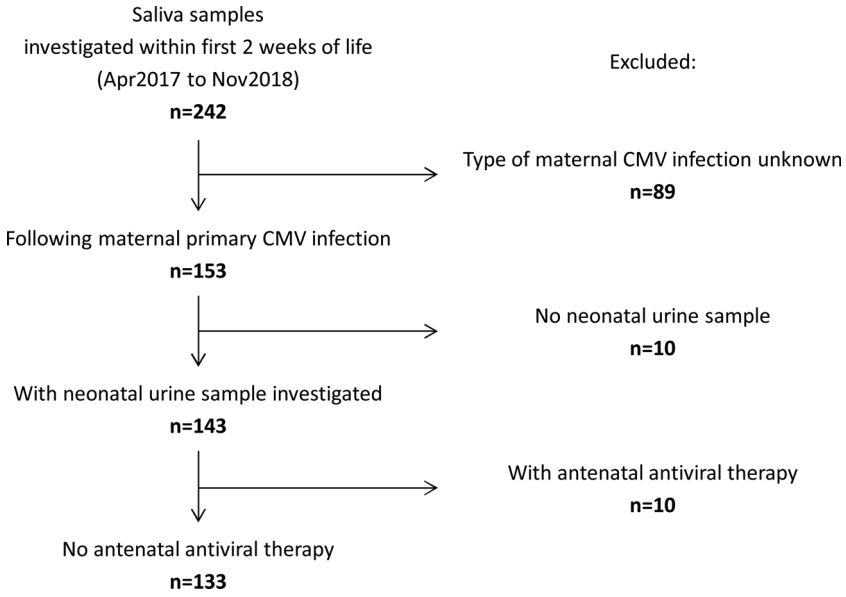

Fig. 1. Selection of study cohort.

transport medium (VTM). Dry swabs sent to our laboratory were transferred in VTM upon receipt. DNA was extracted from 200 µl specimen using MagNA Pure 96 (Roche). CMV DNA was amplified with the RealStar® CMV PCR Kit 1.0 (altona Diagnostics). Low positive PCR results were routinely repeated in cases with discordant findings in urine and saliva. Diagnostic value of PCR was assessed using initial test results. Infectious virus was detected in urine by rapid culture.

4. Results

Between April 2017 and November 2018 we identified saliva-urine pairs of 133 newborns following maternal primary CMV infection in pregnancy, who did not receive antenatal antiviral treatment (Fig. 1). Neonatal saliva swabs and urine samples were obtained within 9 and 12 days of life, respectively. Eighty-six percent (115/133) of saliva samples were collected at the first day of life. Interval between sampling of saliva and urine was 0–8 days (median 0, IQR 0–1), and the time lap between sample collection and receipt in the laboratory was median 3 days (IQR 2–4). Eighty percent (n = 106) of saliva samples were rayon-tipped applicators placed in VTM, the remaining were dry swabs (n = 14) or other swabbing materials (n = 13). In 55% (73/133) of infants CMV PCR was also performed in cord blood. Twenty-nine children were diagnosed with cCMV based on combined results of PCR in urine, saliva and cord blood and rapid culture in urine. Table 1 presents the diagnostic value of saliva PCR compared to urine PCR. Concordantly positive and negative CMV PCR results in urine and saliva were observed in 27 and 94 newborns, respectively (overall concordance: 91% 121/133). CMV DNA detection in cord blood, if available, confirmed these results (25 positive, 40 negative). Rapid culture in urine was performed in 117 of 121 newborns with concordant saliva-urine pairs and results were consistent (23 positive, 93 negative) in all but one case (CMV-DNA positive in urine, saliva and cord blood; rapid culture negative). In concordantly positive samples, median viral load was significantly higher in urine than in saliva ($p = 0.0143$; median CT value 18.47 vs. 20.62; n = 27) or cord blood ($p < 0.0001$; median CT value 18.31 vs. 30.18; n = 25), Wilcoxon Test, Fig. 2.

The 12 cases with discordant PCR results in saliva-urine pairs are presented in Table 2. Congenital CMV was diagnosed in cases 1 and 2 and excluded in cases 3–12 based on results in urine and/or cord blood. cCMV infection in the first two cases was also confirmed by a second urine sample (data not shown). In total we observed 2 false-negative and 10 false-positive PCR results in saliva (all collected at first day of life). PCR re-testing of the 10 false-positive saliva samples confirmed low positive results (CT > 30) in 5 of them, whereas the remaining samples were negative. All 10 newborns were vaginally delivered. Viral load in false-positive saliva samples was significantly lower ($p < 0.0001$; Mann-Whitney test; median CT value 38.45) than in the 27 true-positive saliva samples (median CT value 20.62).

Table 1
Diagnostic value of CMV-DNA detection in saliva (PCR) compared to urine (PCR) for diagnosis of cCMV in newborns following maternal primary CMV infection (n = 133).

		Urine (PCR)			Sensitivity	Specificity	PPV	NPV
		Positive	Negative	Total	% [95% CI]	% [95% CI]	% [95% CI]	% [95% CI]
Saliva (PCR)	Positive	27	10	37	93 [77–99]	90 [83–95]	73 [56–86]	98 [93–100]
	Negative	2	94	96				
	Total	29	104	133				

CI: confidence interval; NPV: Negative predictive value; PPV: Positive predictive value.

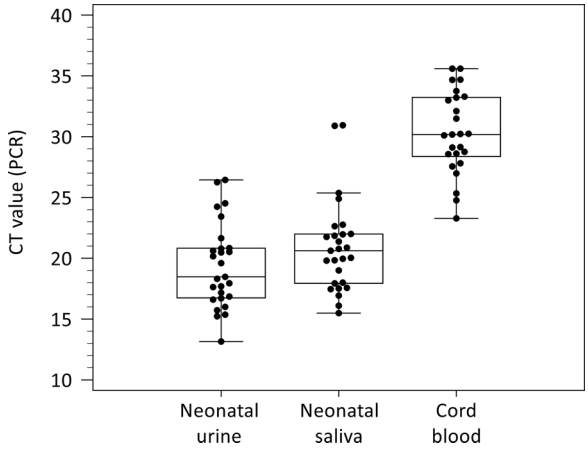

Fig. 2. CT (cycle threshold) values in samples of newborns following maternal primary CMV infection with concordant positive PCR results in urine and saliva (n = 27) and cord blood (n = 25).

5. Discussion

In our cohort of newborns following maternal primary CMV infection, detection of CMV DNA in neonatal saliva showed a lower diagnostic value compared to urine. In two infants we observed false-negative saliva PCR results. In one case (no. 1, Table 2) the mother delivered at gestational week 38 and was diagnosed with primary infection at gestational week 34 (CMV IgG II Liaison XL, Diasorin: 33.1 U/ml, CMV IgM II Liaison XL, Diasorin: 72.4 U/ml, Vidas CMV IgG Avidity II, BioMérieux: 0.24 low avidity). It is likely that late intrauterine transmission accounts for the very low viral load in neonatal urine and cord blood and the negative finding in saliva. The false-negative saliva result in case no. 2 is most likely attributable to a pre-analytical error (e.g. insufficient volume of saliva by incorrect sampling). However, we cannot definitely determine the cause of the false negative result, since the RealStar® CMV PCR Kit 1.0 contains no endogenous internal control for presence of human DNA in the sample.

We observed 10 false-positive CMV PCR results in saliva resulting in a positive predictive value (PPV) of 73%. These results were all low positive (CT values > 30) and did not overlap with the range of CT values found in the 27 saliva samples of newborns considered as true-positive. This is in accordance with other studies, showing that viral load in saliva of infants with cCMV was significantly higher than in uninfected infants with false-positive saliva results [3,4]. In those large-scale screening studies the PPV of saliva PCR was 92.5% (284/307) [3] and 58.6% (51/87) [4], respectively. Both studies did not compare PCR results of saliva to concomitantly obtained neonatal urine (gold standard). Unfortunately, a negative predictive value could not be assessed, since confirmatory testing was only performed in infants with positive saliva results. An earlier study on newborn screening comparing urine with saliva, identified four false-negative but no false-positive saliva PCR results [2].

Potential sources for contamination of neonatal saliva samples are breast feeding or contact with CMV-containing secretions in the maternal genital tract of CMV-seropositive women during birth. It is advised to take saliva samples immediately before [5] or at least one hour after breastfeeding [1,6]. In our cohort all 10 false-positive saliva samples were collected from newborns after vaginal delivery at first day of life. Information on exact time of sampling (before or after first breast feeding) was not available. However, assuming that the prevalence and/or quantity of CMV DNA in colostrum at first day of life are low, it is more likely that false-positive results in saliva in our study are due to contamination from genital secretions than by breast milk. One may speculate that genital shedding occurs more frequently or with higher viral load in women following primary infection during pregnancy, which would explain our relatively high rate of false-positive saliva samples.

The strength of our study is the investigation of concomitantly obtained neonatal saliva and urine sample of pregnancies with primary CMV infection. A limitation is the lack of standardization with respect to sample collection, swab devices and time of sampling. Noteworthy, a recent study demonstrated a good recovery of CMV DNA from various swabbing materials (including dry swabs and swabs in VTM) and observed that duration of storage has no major effect on recovery efficiency [7].

For neonatal screening, saliva has undeniable practical advantages compared to urine, but is prone to sampling errors. The latter may lead to false-negative results and infected newborns could be overlooked and lost to follow-up. Therefore, if the diagnosis of cCMV infection is solely based on saliva testing, PCR assays should contain an endogenous internal control for validation of correct sampling. Furthermore our data support the current recommendation that all positive saliva PCR results have to be confirmed by timely investigation of an urine sample [5]. In pregnancies complicated by primary CMV infection the prevalence of cCMV is much higher than in a screening cohort. Parents are waiting for results of CMV diagnosis at birth with particular concern and the method with the highest diagnostic accuracy should be applied. Therefore in our opinion in this setting detection of CMV by PCR in neonatal urine remains the gold standard for diagnosing cCMV infection.

Table 2
Detailed results on 12 cases with discrepant results of CMV detection in saliva (PCR) compared to urine (PCR).

Case number	Urine PCR		Saliva PCR		Cord blood PCR		Urine rapid culture
	Result	CT value	Result	CT value	Result	CT value	Result
1	low pos.[a]	36.21	negative	–	low pos.	35.35	negative
2	positive	20.26	negative	–	positive	29.50	high pos.
3	negative		low pos.[b]	41.89	negative		negative
4	negative		low pos.[a]	35.25	negative		negative
5	negative		low pos.[a]	39.82	negative		negative
6	negative		low pos.[b]	37.83	na		negative
7	negative		low pos.[a]	34.23	negative		negative
8	negative		low pos.[b]	39.63	na		negative
9	negative		low pos.[b]	39.58	na		negative
10	negative		low pos.[a]	37.93	negative		na
11	negative		low pos.[b]	38.96	na		negative
12	negative		low pos.[a]	37.90	negative		negative

[a] CMV-DNA positive at re-testing.
[b] CMV-DNA negative at re-testing; na: not available.

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Competing interests

None declared.

Ethical approval

Not required.

CRediT authorship contribution statement

Simone Exler: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Anja Daiminger:** Formal analysis, Writing - original draft, Writing - review & editing. **Michaela Grothe:** Investigation, Data curation. **Gunnar Schalasta:** Validation, Supervision, Writing - review & editing. **Gisela Enders:** Writing - review & editing. **Martin Enders:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

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