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Pooling Saliva Sample as an Effective Strategy for the Systematic CMV Screening of Newborns—A Multicentric Prospective Study

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Background: Cytomegalovirus is the most common cause of congenital infections worldwide. Screening all newborns in the first 2 weeks of life is the only way to detect all cases of congenital infection, allowing the monitoring of children with asymptomatic infection at birth and early intervention.

Aim: In this multicenter study, we aimed to evaluate the feasibility of using a saliva pool strategy for mass screening in 7 Portuguese hospitals, and to estimate the current prevalence of this congenital infection in these hospitals.

Methods: A total of 7033 newborns were screened between June 2020 and June 2022, and 704 pools of 10 saliva samples were analyzed by polymerase chain reaction (PCR).

Results: Of the 704 pools analyzed, 685 were negative and 19 had positive PCR results for cytomegalovirus. After individual PCR testing, 26 newborns had positive saliva results, of which 15 were confirmed by urine testing. Thus, this study's prevalence of congenital infection was 0.21% (95% confidence interval: 0.12%–0.35%).

Conclusions: In this study, the pooling strategy proved to be effective for the systematic screening of newborns, although this low prevalence raises questions regarding the cost-effectiveness of implementing universal screening. However, this prevalence is probably the result of the control measures taken during the pandemic; therefore, the rates are expected to return to prepandemic values, but only a new study after the pandemic will be able to confirm this.

Key Words: CMV, congenital, screening, pool

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Cytomegalovirus (CMV) is the most frequent cause of congenital infections worldwide, with a prevalence ranging from 0.2% to 2.2%. In industrialized countries, an estimated 0.6% to 0.7% of newborns are congenitally infected with this virus, which is now the major infectious cause of sensorineural hearing loss and neurodevelopmental defects in infants born in these countries. Long-term sequelae of this infection affect a larger number of children than other known clinical conditions, such as Down syndrome, fetal alcohol syndrome and spina bifida.[1–3]

Currently, maternal screening during pregnancy is not recommended, although there is some hope that antenatal treatment may be approved soon.[4] However, it is important to emphasize that prenatal screening only targets primary infections, which means that a large proportion of cases of congenital infection will go undetected, even with the universal prenatal screening program.[5]

Between 85% and 90% of newborns with congenital infection are asymptomatic at birth, but 10% to 15% of these newborns may develop late sequelae, mainly neurosensorial hearing loss, decreased visual acuity and other neurological changes.[6] Screening all newborns in the first 2 weeks of life is the only way to detect all cases of congenital infection.[7] Implementing this procedure would enable the monitoring of children with asymptomatic infections at birth, with the proven benefit of early intervention.[8]

In recent years, our team has focused on the prevalence of this infection in Portugal and on disclosing ways to simplify and turn attractive and cost-efficient screening programs for CMV congenital infection. It started with the idea of adapting the principle of pool testing used in blood banks to detect CMV infections. In 2005, our team evaluated the feasibility of pool testing in urine samples, and compared to the gold standard methodology, viral culture and the results obtained supported the use of the urine pool methodology analyzed by PCR for the diagnosis of this congenital infection. In 2012, our team performed a preliminary study using the urine pool methodology for CMV screening. It was shown to be cost-effective since with the low prevalence of congenital infection, only a small number of pools had to be tested individually, and in the negative pools, all the samples included were negative, reducing time consumption.[9] However, as other teams have reported,[10]

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All relevant data are within the paper and its Supporting Information files.The study was approved by the ethics committees of all the participating hospitals. Written informed consent was obtained from the parents for newborn enrollment in the study.

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the collection of urine samples has inherent difficulties; therefore, in 2020, our group tested the pool methodology in saliva samples. Saliva samples are much easier to collect, and this pool methodology has similar sensitivity and specificity when compared to individual PCR, allowing a significant reduction in reagent cost and execution time.[11]

Later that year, our group started a larger-scale screening project, the current project, to evaluate the feasibility of using this saliva pool strategy for mass screening in 7 Portuguese hospitals. We also aimed to estimate the current prevalence of this congenital infection in the newborns of the participating hospitals.

MATERIALS AND METHODS

Population

The population of this study included newborns (between 1 and 10 days old) from 6 hospitals in the Lisbon area (Hospital CUF Descobertas, Hospital Vila Franca de Xira, Hospital S. Francisco Xavier-CHLO, Hospital da Luz de Lisbon, Hospital dos Lusíadas de Lisbon and Maternidade Alfredo da Costa) and one in Oporto (Hospital CUF Porto). The samples were collected between June 2020 and June 2022 (Fig. 1). All newborns born in these hospital maternities were included, except when parental permission was not obtained.

Samples

Saliva samples were collected using breakable rod rayon swabs (FLmedical, Torreglia, Italy), and were collected before breastfeeding or at least 1 hour after the last meal. The swab was packaged in a 3 mL tube with 750 µL of Roswell Park Memorial Institute 1640 cell culture medium medium (Life Technologies, Paisley, United Kingdom). After collection, tubes were stored at 4 °C until further processing. When a positive result was obtained with a saliva sample, a urine sample was collected in the first 2 weeks of life to confirm congenital infection. For this purpose, fresh urine samples were collected, placed in 50 mL flasks and stored at 4 °C until processing.

All samples were sent to the Laboratory of Microbiology associated with the Infection Unit of the Faculdade de Ciências Médicas|Nova Medical School for processing.

Sample Processing

Sample processing has been described in detail elsewhere.[12] Briefly, pools were prepared by mixing 20 µL of each of the 10 saliva samples in a 1.5 mL Eppendorf tube. Genomic DNA was extracted using the Purelink Genomic DNA commercial kit (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions, and stored at −20 °C until use. CMV DNA was amplified and detected by quantitative PCR on the Applied Biosystems 7500 Fast Real-Time PCR System 116 (Applied Biosystems, Foster City, CA) using an "in-house" method. When a pool was positive, each sample was tested individually. Upon identification of the positive sample(s) to confirm congenital human cytomegalovirus infection, a urine sample was requested and tested using the extraction and PCR methods described above.

RESULTS

The total number of newborns involved in this project was 7033, with the following distribution per hospital: Hospital S. Francisco Xavier-CHLO (n = 2225), Hospital Vila Franca de Xira (n = 1352), Hospital CUF Descobertas (n = 1059), Hospital dos Lusíadas de Lisbon (n = 810), Hospital da Luz de Lisbon (n = 637), Hospital CUF Porto (n = 544) and Maternidade Alfredo da Costa (n = 406).

To screen 7033 newborns, 704 sample pools were analyzed: 685 were negative, and 19 had positive PCR results for CMV. After individual PCR testing, 26 newborns had positive saliva results, of which 15 were confirmed by urine testing (Fig. 2). Thus, this study's prevalence of congenital infection was 0.21% (95% confidence interval: 0.12%–0.35%). Considering only the hospitals in the Lisbon area, the prevalence was 0.23% (15/6489; 95% confidence interval: 0.12%–0.35%).

DISCUSSION

Universal screening for CMV in newborns remains controversial. Before becoming established, it is necessary to weigh the benefits versus harms. The potential benefits of newborn CMV screening include early intervention addressing hearing loss, cognitive deficits and vision impairment, including antiviral drugs. Nonpharmaceutical therapies, such as speech-language therapy or

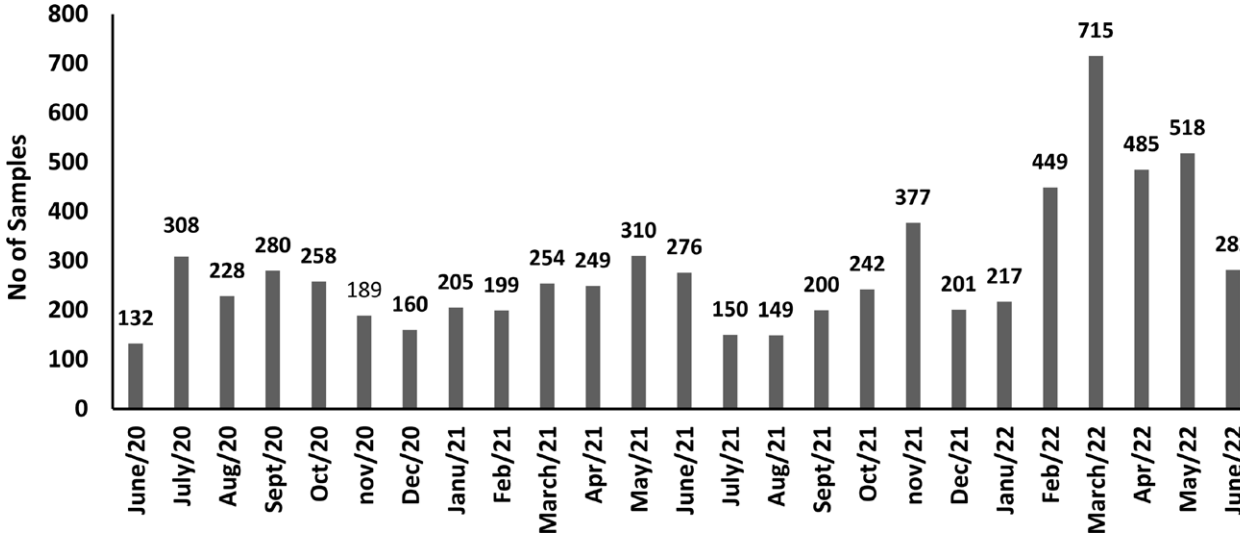

FIGURE 1. Monthly distribution of newborn saliva samples.

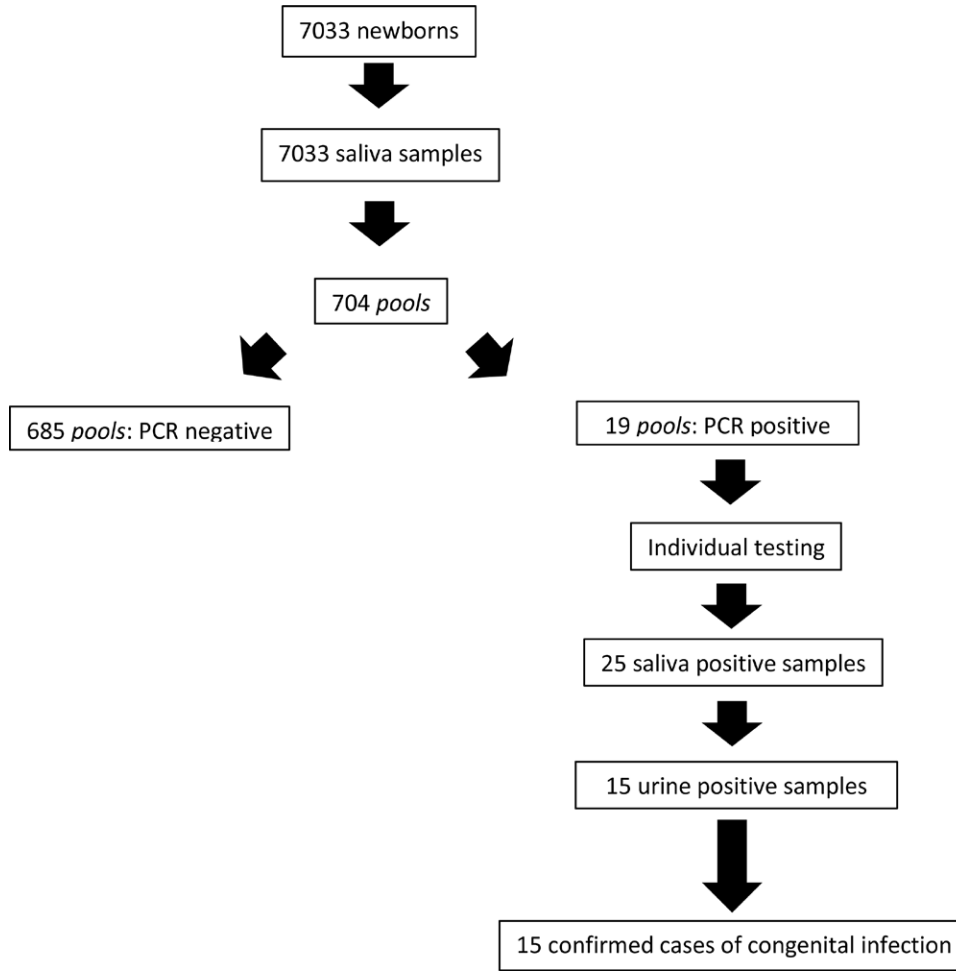

FIGURE 2. Algorithm and results of the pooling strategy.

cochlear implants can be indicated in cases of hearing impairment. Evidence suggests that these interventions can improve clinical outcomes. However, potential harm may include increased parental stress from a false positive, or even a true positive screening result (many truly positive children will never develop sequelae), inappropriate antiviral treatment or added costs from unnecessary medical visits or tests.[13]

A crucial point for this discussion is the feasibility of a screening program for all newborns, from both an economic point of view and the burden of laboratory work. Our pool strategy, with excellent agreement with individual testing, significantly reduces the cost of both testing and laboratory workload.[11] In the current study, we proved that screening a large group of newborns from different hospitals was feasible, with the pool testing centralized in just 1 laboratory.

The major problems faced with this strategy were neither linked to the overload of laboratory workload nor the transport of samples from the hospitals to the laboratory, even though one of the hospitals was 300 km away from the laboratory. Regarding the first, overload of laboratory work, 1 technician spending a few hours daily, was enough to process the samples from all participating hospitals. However, we must admit that the low prevalence found in this project contributed to fewer positive pool confirmations; thus, the workload was lower. Nevertheless, with prevalence values obtained in Portugal before the pandemic,[11] only 1 or 2 in 20 pools will have to undergo individual PCR, so the workload will be greatly reduced.

At the beginning of this study, we noticed a very low prevalence (0.078%), which was attributed to the effects of the pandemic.[14] Thereafter, the lifting of some restrictions may be at the origin of a slight increase to 0.23%, the final rate obtained in the current study, but still below what would be expected for Portugal, considering previous studies conducted in this country before the pandemic.[11]

Therefore, the 2 main conclusions of this study point in different directions: the pooling strategy proved to be effective for the systematic screening of newborns and should be considered as a potential solution to overcome the burden of both the cost of testing and laboratory workload. Conversely, the low prevalence rate found in this study raises questions regarding the cost-effectiveness of universal screening for this infection. However, this prevalence is probably the result of the control measures taken during the pandemic; therefore, the rates are expected to return to prepandemic values, but only a new study after the pandemic will be able to confirm this.

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