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Neonatal screening parameters in infants with congenital *Cytomegalovirus* infection

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ABSTRACT

Congenital *Cytomegalovirus* infection (cCMV) is the most common cause of congenital infections worldwide that can cause long-term impairment (LTI). The metabolic alterations due to cCMV are largely unknown. This study aims to assess the metabolites included in the neonatal screening in relation to cCMV and cCMV outcome, allowing the identification of prognostic markers for clinical outcome. Essential amino acids, hormones, carnitines and enzymes from Dried Blood Spots (DBS) were analyzed of 102 children with cCMV and 179 children without cCMV, and they were related to symptoms at birth and LTI at 6 years of age. In this cohort, the neonatal screening parameters did not change in relation to cCMV, nor to symptoms at birth or LTI. However, metabolic changes were observed in children born preterm, with lower concentrations of essential amino acids in premature infants with cCMV compared to premature controls. Finally, a higher concentration of palmytoilcarnitine (C16) in the group with higher viral load was observed. Though these data demonstrate limitations in the use of neonatal screening data as predictors for long-term cCMV outcome, the metabolism of preterm neonates with cCMV merits further evaluation.

1. Introduction

Cytomegalovirus (CMV) infection is a common infection with a seroprevalence of almost 50% in the general Dutch population [1]. CMV is the most common cause of congenital infections worldwide with an overall birth prevalence of 0.6–0.7% in industrialized countries [2,3]. A significant part of children with congenital CMV infection (cCMV) will have long-term permanent neurological sequelae. Among congenitally CMV-infected children, 12.7% is estimated to be symptomatic at birth with the most common symptoms being petechiae, jaundice, hepatosplenomegaly, thrombocytopenia, chorioretinitis, and microcephaly [2,3]. An estimated 40–58% of these symptomatic children will develop permanent sequelae, such as hearing loss, mental retardation, and developmental delay [3]. Approximately 13.5% of the asymptomatic children will develop permanent sequelae as well [3].

cCMV outcome is the result of a complex interplay between viral, maternal, fetal, and child factors. *In vitro*, CMV has been shown to influence different cellular metabolic pathways. The fatty acid biosynthetic pathway of infected cells is highly upregulated in order to sustain the viral envelope production [4–6]. In infected cells, the increased glucose uptake and glycolytic activity provide the necessary carbon atoms used for fatty acid biosynthesis [4,6,7]. The cellular energy requirement is then insured by the increase of glutaminolysis in order to allow the tricarboxylic acid cycle (TCA) to function [8]. *In vivo*, few studies have evaluated the metabolic changes occurring in children with cCMV. A recent metabolomics analysis on amniotic fluid (AF) showed that primary CMV infection during pregnancy, irrespective of fetal infection, resulted in the activation of glutamine-glutamate and pyrimidine metabolic pathways and, when comparing asymptomatic CMV-infected newborns to symptomatic CMV-infected newborns, a possible shift in fatty acid biosynthesis was observed [9]. Moreover, in a group of congenitally infected children a metabolic fingerprint was

Abbreviations: cCMV, congenital *Cytomegalovirus* infection; DBS, dried blood spots; LTI, long-term impairment; C16, palmytoilcarnitine; 17OHP, 17-α-hydroxyprogesterone; T4, thyroxine; AF, amniotic fluid; BIOT, biotinidase activity; MS/MS, Tandem Mass Spectrometry

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Table 1
Genetic disorders included in the Dutch neonatal screening program in 2008.

Disorder	Marker	Quantification method	Incidence[a]
Amino acid disorders			
Glutaricaciduria type 1 (GA I)	C5DC	MS/MS	1: 335,455
Isovaleric academia (IVA)	C2, C5	MS/MS	1: 351,429
Maple syrup urine disease (MSUD)	Leucine, Valine	MS/MS	1:567,692
Homocystinuria (HCU)	Methionine	MS/MS	1:167,727
3-methylcrotonyl-CoA- carboxylase deficiency (3-MCC)	C5OH	MS/MS	1: 194,211
HMG-CoA lyase deficiency	C5OH	MS/MS	1:100,000 [34][b]
Multiple CoA carboxylase deficiency (MCD)	C5OH	MS/MS	1:200,000 [35]
Phenylketonuria (PKU)	Phenylalanine, Tyrosine	MS/MS	1: 11,865
Fatty acid oxidation disorders			
Medium chain acylCoA dehydrogenase deficiency (MCAD)	C8, C10	MS/MS	1: 23,730
Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)	C16OH	MS/MS	1:410,000
Very long chain acylCoA dehydrogenase deficiency (VLCAD)	C14:1, C16	MS/MS	1: 144,706
Carnitine transporter deficiency (CTD)	C0	MS/MS	1:40,000 [36]
Endocrine disorders			
Congenital hypothyroidism (CH)	T4, TSH, TBG	Immunochemistry	1:3000–1:4000 [37]
Congenital adrenal hyperplasia (CAH)	17-OHP	AutoDELFIA	1:10,000–1:20,000 [38]
Other			
Galactosemia (GAL)	GALT, TGAL	Enzymatic method	1: 49,530
Biotinidase deficiency (BTD)	BIOT	Enzymatic method	1: 49,865

[a] Unless otherwise specified the incidence is retrieved from the Dutch Diagnosis Registration Metabolic Diseases (DDRMD) database [39].
[b] For HMG-CoA lyase deficiency the prevalence is reported.

identified in urine samples compared to uninfected controls. An increase of ketone bodies (3-hydroxybutyrate and 3-aminoisobutyrate) was observed in the CMV-infected group in an attempt to compensate a general reduced level of ATP [10].

The aim of this study was to assess the metabolites included in the neonatal screening, which is performed in dried blood spots (DBS), in relation to cCMV and cCMV outcome. Importantly, several considerations should be taken into account with respect to the neonatal screening, specifically designed to diagnose rare genetic metabolic disorders. First of all, excluding the metabolic disorders, changes in these metabolites have only been reported in critically ill children. A decrease in thyroid hormones in septic neonates with poor outcome was observed and premature critically ill neonates showed different amino acids profiles, usually with higher concentrations, compared to the healthy controls [11,12]. The majority of newborns with cCMV do not have symptoms at birth or only have mild disease, and the clinical signs of symptoms and LTI included in this cohort are diverse. Therefore, if any changes in metabolites are found, these will most likely be subtle. Second, several factors have been described influencing the analytes measured on DBS, such as fetal blood volume, hematocrit, gestational age, birth weight, maternal factors and storage conditions [13–19]. However, despite these potential limitations, this exploratory study was undertaken to study biomarkers in neonatal DBS of cCMV-infected children. This could allow the identification of prognostic markers for long term outcome of cCMV with a profound impact on parental counselling, postnatal interventions and the potential introduction of neonatal screening for cCMV. For this purpose, the neonatal screening data of a large nation-wide cohort of children with and without cCMV was evaluated in relation to long-term impairment (LTI) at the age of six years.

2. Materials and methods

2.1. Study population and clinical data

A previously described, nationwide, retrospective cohort was used for this study. A group of 31,484 children born in 2008 in the Netherlands was retrospectively tested for cCMV by PCR for CMV DNA in neonatal DBS at 5 years of age [20]. cCMV was diagnosed in 156 children and informed consent for retrieval of medical data was given by parents of 133 children with cCMV and 274 matched controls. After approval by the Medical Ethics Committee of the Leiden University Medical Center, the parents of 102 congenitally CMV-infected children and 197 children without cCMV gave informed consent to retrieve the neonatal screening data of their child. The controls without cCMV are from a gender-, month-of-birth and region-matched control group. Children were defined as symptomatic at birth if they had one or more of the following signs or symptoms in the neonatal period: prematurity, being small for gestational age, microcephaly, hepato- or splenomegaly, generalized petechiae or purpura, hypotonia, abnormal laboratory findings (elevated liver transaminases, hyperbilirubinemia, neutropenia or thrombocytopenia), cerebral ultrasound abnormalities, ophthalmologic abnormalities or neonatal hearing impairment. LTI was defined as the presence of impairment in one or more domain (hearing, visual, neurological, motor, cognitive and speech-language). Because in this cohort maternal seroimmunity to CMV before birth was unknown, it was assumed that cCMV infection could have resulted from either maternal primary or recurrent infection.

2.2. DNA extraction from DBS and qPCR of CMV

After a first initial CMV PCR screening performed at the National Institute for Public Health and the Environment (RIVM), a second confirmatory PCR was performed at the Leiden University Medical Center (LUMC) [20]. For this purpose, DNA was extracted from DBS by using the QIAamp DNA minikit according to the previously described protocol [21]. For each test one full DBS was punched by using an automated DBS puncher (1296-071, Perkin Elmer-Wallac, Zaventem, Belgium). CMV DNA amplification of a 126-bp fragment from the immediate-early antigen region was performed using an internally controlled quantitative real-time PCR as described previously [22,23] on a CFX96 Real-Time PCR Detection System (BioRad, Veenendaal, The Netherlands). The PCR was performed in triplicate, and the CMV viral load expressed in IU/ml.

2.3. Neonatal screening data

The genetic metabolic disorders included in the Dutch neonatal screening program in 2008 are listed in Table 1. The screening is carried out on DBS and involves five regional accredited screening laboratories, among which the National Institute for Public Health and the Environment (RIVM). The DBS are collected between 72 and 168 h