Predicting Early Vestibular and Motor Function in Congenital Cytomegalovirus Infection

Short running title: Predicting vestibular and motor cCMV outcome

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ABSTRACT

Objectives

Congenital cytomegalovirus (cCMV) can affect the vestibular function, which is an important cornerstone for early motor development. This study aims to identify risk factors for early vestibular dysfunction with severe repercussions on motor outcome.

Methods

This prospective cohort study included 169 cCMV-patients with complete vestibular assessment (lateral video Head Impulse Test and cervical Vestibular Evoked Myogenic Potentials) before the age of 18 months (mean 8.9, SD 3.27 months). Motor results using the Alberta Infant Motor Scale were collected in 152 of these patients. Logistic and linear regression models were applied to identify risk factors for vestibular and motor outcome, respectively.

Results

The odds of developing early vestibular dysfunction were 6 times higher in patients presenting with hearing loss at birth compared to those born with normal hearing ($p = 0.002$). Within the latter group, significant predictors for vestibular dysfunction were (delayed-onset) hearing impairment at the time of vestibular testing ($p = 0.003$) and the presence of periventricular cysts on magnetic resonance imaging ($p = 0.005$). Remarkably, none of the patients infected during the third trimester of pregnancy (n=14) developed early vestibular dysfunction. On average, vestibular-impaired patients had a z-score on the Alberta Infant Motor Scale that was 1.17 points lower than patients without vestibular deficit ($p < 0.001$).

Conclusion

Early vestibular loss can have a significant adverse effect on motor development. Hearing and cranial imaging findings could facilitate the widespread implementation of a (targeted) vestibular assessment approach in the cCMV-population.

Short summary: This study shows the impact of dysfunction in the balance organ on early motor development in infants with congenital cytomegalovirus infection (cCMV) and identifies predictors for this balance loss, which could facilitate widespread implementation of balance assessment in cCMV.

Keywords: Congenital cytomegalovirus; vestibular; motor; predictor; paediatric

Level of evidence: 3

INTRODUCTION

Affecting 0.2 to 6.1% of all live births, congenital cytomegalovirus (cCMV) is the leading congenital infection worldwide and an important cause of neurodevelopmental impairment in children[1, 2]. Clinical presentation of infants born with cCMV is heterogeneous, ranging from severely symptomatic to (most commonly) completely asymptomatic. Around 20% of all infected infants develop long-term sequelae[3, 4]. Sensorineural hearing loss (SNHL), the most common sequela in cCMV, is reported to be caused by a viral labyrinthitis potentially combined with detrimental host immune responses[5-7]. Although it has long remained in the obscurity, growing evidence shows that not only auditory, but also vestibular function can be affected[8-19]. As vestibular input is an important cornerstone for adequate early motor development, timely detection of vestibular loss could be important to reach a child's full potential[20-24]. Research suggests that cCMV-related vestibular dysfunction primarily occurs in children with SNHL, but also normal-hearing asymptomatic children can be affected[8, 10, 12, 13, 16, 19]. Moreover, similar to cCMV-related SNHL[25-28], cCMV-related vestibular loss appears to be unpredictable in onset and severity[10, 13, 19]. Timely detection of vestibular deficits would imply longitudinal vestibular follow-up for the entire (large) cohort of cCMV-infected patients, an approach that involves considerable burden for both diagnostic centres and patients. Therefore, through analysis of prenatal, perinatal and postnatal data, this study aims to identify predictive factors for cCMV-patients at risk for vestibular dysfunction with significant repercussions on motor outcome.

MATERIALS AND METHODS

cCMV-protocol

In Flanders (Belgium), patients are tested for cCMV after known maternal seroconversion during pregnancy, cCMV-like symptoms in the child or cCMV-diagnosis after neonatal

screening (offered in certain hospitals). Diagnosis of cCMV is based on virus isolation or CMV-DNA positive polymerase chain reaction (PCR) in urine or saliva sample taken within the first 3 weeks of life (n=163) or by retrospective analysis through CMV-DNA positive PCR on dried blood spot (n=6). To rule out contamination by breast milk, positive PCR in saliva is confirmed by PCR on urine, also taken before 3 weeks of age. Confirmed cCMV-patients in our centre undergo neonatal assessment consisting of clinical examination, blood tests, cranial ultrasound (US) and magnetic resonance imaging (MRI), ophthalmologic examination and neonatal hearing screening followed by diagnostic auditory brainstem response (ABR) measurement in case of refer. Based on these results, severity of the cCMV-infection is determined (see Supplementary Table 1 for the criteria of classification)[29, 30]. Children with retrospective cCMV-diagnosis after delayed-onset hearing loss are classified as asymptomatic. Hearing-impaired children are considered severely symptomatic. Postnatal antiviral therapy is proposed in case of moderate or severe symptomatic cCMV with the exception of patients with isolated bilateral severe or profound SNHL. For all information on our cCMV-work up, see Keymeulen et al. (2021)[30].

Subjects

The study was approved by the Ghent University Hospital ethical committee (EC UZG 2015/1441), and informed consents were asked from the children's parents. Since the start of standard vestibular follow-up for all cCMV-children in our centre in 2016, 217 subjects have been enrolled of which 169 and 152 subjects were included for vestibular and motor analyses, respectively. Details on our exclusion criteria can be found in Figure 1.

Auditory, vestibular and motor assessment

All confirmed cCMV-patients are enrolled in 4-year longitudinal auditory, vestibular and motor follow-up[10, 31]. Apart from the neonatal hearing evaluation, the most recent click-ABR

measurement (free from middle ear effusion) before the selected vestibular assessment was included for analysis[31]. In children younger than 6 months, ABR measurements were performed in natural sleep. Beyond that age, ABR was performed under anaesthesia or melatonin-induced sleep. Click-thresholds > 30 dB nHL were classified as hearing impairment. Focusing on early development, only the most reliable and complete vestibular assessment before the age of 18 months was included in this analysis. This included reliable completion of both the video Head Impulse Test (vHIT) for lateral semicircular canals (SCC) and cervical Vestibular Evoked Myogenic Potentials (cVEMP). In case of early progressing vestibular deficit, the most severely affected measurement before 18 months of age was included. Details on the vestibular assessment protocol are described in Dhondt et al. (2020)[10].

vHIT for lateral SCC evaluation was performed using a stand-alone camera in front of the child (vHIT Ulmer version 3.5.0.6, Synapsys, Marseille, France). vHIT manoeuvres were performed with an amplitude between 10 and 20 degrees and peak velocity above 150 degrees per second. Gain values between 0.7 and 0.4 were considered mild lateral SCC dysfunction[32] and values below 0.4 were defined as severe impairment[10]. Saccular function was evaluated through bone conducted cVEMP measurements (Bio-Logic Navigator- Pro platform, Mundelein, IL, USA and Neuro-Audio version 2010, Neurosoft, Ivanovo, Russia) using linear 500 Hz tone bursts presented at the mastoid at 59 dB HL (129 dB FL) intensity. Rectified interpeak amplitudes below 0.3 (Bio-Logic) or 1.3 (Neuro-Audio) were classified as mild saccular dysfunction[32]. Severe saccular dysfunction was concluded if no reproducible cVEMP-response could be elicited at all[10]. Patients with a SCC and/or a saccular dysfunction (mild or severe) were classified as having vestibular loss.

Gross motor development was evaluated by means of the Alberta Infant Motor Scale (AIMS), an observational measure examining gross motor skills from birth until independent walking. Total raw scores were converted to age-corrected z-scores based on a Belgian standardization

group[33]. Z-scores below -2 represent weak gross motor performance. Extremely low z-scores (<-4, n=3) were entered as -4 in the database.

Statistical analysis

Descriptive statistics were computed to display occurrence and characteristics of inner ear dysfunctions. Continuous outcome parameters between subgroups were compared by means of Kruskal-Wallis tests with Dunn's post hoc analyses with Bonferroni correction. Logistic and linear regression models were applied to identify significant predictors for (1) vestibular function (normal or abnormal, i.e. categorical outcome) and (2) gross motor performance (AIMS z-score, i.e. continuous outcome), respectively. Odds ratio's (OR, logistic regression) and regression coefficients (linear regression) with 95% confidence intervals (CI) and *p*-values were interpreted. For prediction of both outcomes, univariable analysis for a first predictor in the complete subject group preceded the building of a final multivariable model in a subgroup of patients selected by this first variable. This approach was necessary to bypass possible multicollinearity between independent variables. Potential predictors were selected on the basis of clinical and scientific knowledge to avoid overfitting. (1) For vestibular outcome, predictive value of trimester of maternal seroconversion, perinatal risk (gestational age < 37 weeks and/or birth weight < 2500g or not), severity of the cCMV-infection (severely affected or not), abnormalities on imaging (see further) and hearing status at birth and at the time of vestibular testing (normal or abnormal) were assessed. After univariable analysis in the complete subject group (n=169) with hearing status at birth as predictor, further multivariable analyses were performed in the subgroup of patients with normal hearing at birth (n=149). Subsequently, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for significant predictors. (2) For motor outcome, predictive value of gestational age (continuous), abnormalities on imaging (see further), hearing status at the time of vestibular testing (normal or abnormal), cochlear implant (CI) status at the moment of

vestibular testing (CI or not) and vestibular function (normal or abnormal) were studied. After univariable analysis in the complete motor dataset (n=152) with vestibular function as predictor, further multivariable analyses were performed in the subgroup of patients with normal vestibular function (n=135). To determine which specific brain abnormalities were predictive for vestibular or motor outcome, all abnormalities that occurred in minimal 3 patients (Table 1) were included in a multivariable analysis (US and MRI separately). Subsequently, abnormalities with significant ($p < 0.05$) predictive value were added to the final multivariable models. SPSS software (IBM, version 27.0, Armonk, NY) was used for all analyses.

RESULTS

Subject characteristics

Children's average age at the included vestibular examination was 8.9 (SD 3.27) months. The mean interval between hearing and vestibular assessment was 4.6 (SD 3.02) months. Motor assessment was usually scheduled combined with the vestibular examination (mean interval 0.1 months, SD 0.44 months). More details on subject characteristics are provided in Tables 1 and 2.

Inner ear impairment

SNHL and vestibular dysfunction occurred in 14.2% and 11.8% of all subjects, respectively. Details on the laterality, onset and severity can be found in Table 3. Patients with CI were not excluded as these include an important subgroup of the cCMV-population and previous research of ours has shown that the impact of CI on vestibular function in our centre is very limited[34]. At the moment of vestibular testing, 6 (3.6%) patients already had CI (4 unilateral, 2 bilateral). In 4 out of 8 ears with CI, vestibular dysfunction was detected. Two of these ears were vestibular impaired already before implantation.

Predicting vestibular outcome

Within the complete vestibular dataset (n=169), univariable logistic regression showed that hearing status at birth was significantly predictive for vestibular outcome; the odds of vestibular dysfunction being 6 times higher (p = 0.002, OR = 5.63, 95% CI = [1.91 – 16.60]) for children with SNHL at birth. Within the subgroup of patients with normal hearing at birth (n=149), multivariable models with abnormalities on cranial imaging revealed significant predictive value for the occurrence of periventricular cysts on MRI only, so that this variable was added to the final multivariable model (Table 4). The final model further showed that, apart from periventricular cysts on MRI, (delayed-onset) SNHL at the moment of vestibular testing (i.e. 8.9 months on average) appeared to be a significant predictor for vestibular outcome. Perinatal risk and severe cCMV-infection did not significantly affect vestibular outcome in our data (Table 4). Trimester of seroconversion could not be added to the multivariable model because of complete separation as vestibular dysfunction did not occur in patients with third-trimester seroconversion (n=14). Vestibular dysfunction did occur in 14.3% (8/56) of the patients infected in first trimester of pregnancy, 5.3% (2/38) of those infected in second trimester and 16.4% (10/61) of patients with unknown timing of seroconversion.

Diagnostic value of each of the significant predictors for vestibular outcome and calculated sensitivity, specificity, PPV and NPV are summarized in Figure 2. As illustrated in Figure 2, 35.0% (7/20) of vestibular-impaired patients had SNHL at birth, and 50.0% ((7+3)/20) had SNHL at the moment of vestibular testing.

Predicting motor outcome

Univariable linear regression within the complete motor dataset (n=152) showed the presence of vestibular dysfunction to have significant negative impact on the AIMS z-score ($p < 0.001$, β = -1.17, 95% CI = [-1.75 – -0.60]). Multivariable models with cranial imaging abnormalities

within the subgroup of patients with normal vestibular function (n=135) did not reveal a significant effect for any of the included abnormalities, so that only the overall presence of any abnormality on cranial imaging was added to the final multivariable model. As presented in Table 4, the multivariable model in the subgroup of patients with normal vestibular function further displayed gestational age to have significant impact on the AIMS z-score. No significant effect for cranial imaging abnormality or having SNHL or CI at the moment of vestibular testing could be found in our motor data.

Figure 3 (left panel) displays motor outcome according to presence of vestibular loss and whether patients displayed abnormalities on the previously mentioned significant predictors for vestibular outcome (SNHL and periventricular cysts on MRI). A significant difference was found only between patients with normal vestibular function and those with vestibular dysfunction and SNHL or periventricular cysts ($p = 0.028$).

The middle and right panel of Figure 3 demonstrate the impact of laterality and onset of vestibular loss on motor outcome. These effects were statistically significant, as compared to patients with normal vestibular function, motor outcome was significantly worse in patients with bilateral vestibular dysfunction ($p = 0.023$) and patients with vestibular dysfunction present from the first examination onwards ($p = 0.001$).

DISCUSSION

Vestibular loss before 18 months of age occurred in 11.8% of our cCMV-cohort, which is in the same order of magnitude as the occurrence of early SNHL (14.2%). Whereas longitudinal auditory follow-up for all cCMV-patients is well-established[25, 35, 36], similar vestibular evaluation approaches are rare. Yet earlier research shows that sensory input from the vestibular system is a keystone for early motor development[20-24]. Particularly in cCMV-patients, who are

already at risk for various disorders with potential repercussions on motor outcome[37-39], the value of vestibular function cannot be underestimated. In line with earlier research of our group[12], current analyses show that in the majority of the cCMV-population without severe neuromotor disorder (Fig. 1), vestibular dysfunction seems to be the factor with the greatest (adverse) effect on early motor performance, especially when it is bilateral (Fig. 3). Interestingly, apart from vestibular dysfunction, other factors such as cranial imaging abnormality, CI or SNHL did not appear to be significantly predictive for motor outcome. Our analyses did show a significant (favourable) impact of gestational age on motor outcome, even after exclusion of prematurely born infants. This relation already has been found in healthy infants[40], and appears to also hold true for the cCMV-population.

The significant adverse effect of vestibular dysfunction on motor outcome in our study demonstrates the necessity to monitor vestibular function as closely as hearing. However, longitudinal vestibular follow-up for all cCMV-patients, as is established for hearing, is a demanding protocol[41]. Identifying predictive factors for vestibular dysfunction to enable a targeted approach could therefore markedly increase the feasibility of vestibular evaluation in the large cCMV-population. Various studies have previously shown that vestibular dysfunctions more commonly occur in patients with SNHL compared to normal-hearing patients[12, 13, 17, 19]. This was also confirmed by our results, the odds for vestibular impairment in children with SNHL at birth being 6 times higher compared to normal-hearing children. As such, vestibular assessment in cCMV-children with abnormal neonatal hearing evaluation seems to be a bare minimum. This approach is also in line with the ideal of the Vestibular Infant Screening – Flanders project (VIS-Flanders), a recent initiative in our country in which vestibular screening is provided for children with confirmed permanent hearing loss after refer on neonatal hearing screening[42-44]. Unfortunately, this approach seems insufficient for the cCMV-population, as vestibular function is not necessarily safeguarded in normal-hearing

cCMV-children[8, 12, 13, 16, 19]. Although the risk is considerably lower, the number of normal-hearing cCMV-patients is much higher than the number of hearing-impaired cCMV-patients. Therefore, normal-hearing cCMV-patients could account for an important proportion of the vestibular-impaired cCMV-population[8]. Indeed, this is the case in our dataset in which 65.0% (13/20) of the vestibular-impaired patients had normal hearing at birth and 50.0% (10/20) still had normal hearing at the moment of vestibular testing (Fig. 2). Therefore, a clinical call arises for predictive factors in this major subset of normal-hearing patients. With the odds of having an early vestibular dysfunction in patients with periventricular cysts on MRI being 14 times higher compared to those without this MRI abnormality, an additional predictive factor appeared in our dataset. This is not surprising since some studies reported similar predictive value of cranial imaging for auditory outcome[26, 45-47]. It is however remarkable that only this specific imaging finding, and not any other anomaly or the overall abnormality on brain imaging was significantly related to vestibular outcome. Possibly, this is related to the overall occurrence of this brain finding in the cCMV-population (Table 1) and/or the timing of development of these brain lesions[48]. Surprisingly, presence of periventricular cysts did not appear to be significantly predictive when only detected on US, which could be due to different sensitivity of both techniques for these lesions. Unfortunately, being a reliable, relatively low-cost technique not necessitating some form of sedation, US is currently more commonly used for brain imaging purposes in neonates[29]. Looking at the diagnostic value of each of the predictors for vestibular outcome (Fig. 2), the added value of cranial MRI looks promising, but has to be confirmed in other samples. In our dataset, the sensitivity increases from 50.0% (10/20 patients detected) when only hearing status is considered to 70.0% (14/20 patients detected) when periventricular cysts on MRI are also taken into account. This is accompanied with only a slight decrease of specificity as 32 patients would be tested compared to 25 when only hearing-impaired patients would be targeted. With only 18.9% (32/169) of the total sample to

be identified as “at risk”, these findings could add to general belief that vestibular assessment in the cCMV-population can be worthwhile. Additionally, also timing of seroconversion appears as an important predictor for vestibular outcome, as is already demonstrated for early auditory outcome[26, 38, 49]. In practice, the strong NPV of a third-trimester seroconversion (Fig. 2), suggests that these patients might be least at risk. Interestingly, a recent study of Chebib et al. (2022)[50] on predicting factors for inner ear function as a whole (auditory and vestibular function considered together) reported similar findings as we did. They found abnormalities on antenatal imaging and first trimester seroconversion to be predictive for inner ear function.

This is the first study to assess risk factors for early vestibular and motor dysfunction in a large cohort of symptomatic and asymptomatic cCMV-patients. Predicting outcome in cCMV, for any developmental domain, is currently still very difficult and remains a major cause of parental concern. Although this study managed to identify several important predictors, 30% (6/20) of vestibular-impaired patients did not show abnormalities on any of these variables and therefore would remain under the radar of the predictors. Reassuringly, in our sample, motor outcome of the (more mildly) vestibular-impaired patients without abnormalities for the identified risk factors was not significantly worse compared to patients with normal vestibular function (Fig. 3, left panel). Besides, our motor data show that especially children with vestibular dysfunction present from the first examination onwards have significantly weaker early motor skills compared to those with normal vestibular function, implying that even a single vestibular assessment at early age could make a substantial difference for a child’s development potential (Fig. 3, right panel). It seems clear that vHIT assessment should be pivotal in this evaluation, given its many advantages for application in the paediatric population[41] and the fact that in our sample, SCC dysfunctions were more common than saccular dysfunctions.

It should be pointed out that in the absence of neonatal vestibular screening, it cannot be ruled out that any of the patients with vestibular dysfunction at first examination had a non-congenital

deficit that occurred prior to the first assessment. Additionally, it is important to stress that the impact of vestibular dysfunctions on the long-term outcome and/or developmental skills remains basis for continued research[51]. Intending to identify any vestibular dysfunction in the context of cCMV would still require longitudinal vestibular follow-up of all cCMV-patients with extensive vestibular assessment[10]. Note that early and proper counselling could be a valuable alternative or addition in this respect.

CONCLUSION

Our data show that vestibular dysfunctions can have a significant adverse effect on motor outcome in cCMV-patients, especially when it is bilateral and/or congenital. Targeted vestibular evaluation approaches using even a single assessment or focusing on at risk populations (e.g. with SNHL or periventricular cysts on MRI), for example, can already make a substantial difference.

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FIGURE LEGENDS

Figure 1. Flowchart of subject inclusion and exclusion. Only patients that gave their consent and had complete vestibular assessment before the age of 18 months were included to evaluate the impact of various factors on early vestibular outcome. Patients with additional risk factors for vestibular function loss were excluded as their vestibular outcome may not only be affected by congenital cytomegalovirus infection but also by an additional confounder. The same line of reasoning was followed for exclusion criteria for motor analyses. Prematurity and neuromotor disorders (defined as a clinical diagnosis of cerebral palsy or the detection of periventricular leukomalacia on cranial imaging) are known to have an important impact on motor outcome, independent of congenital cytomegalovirus infection. Obviously, only patients with available AIMS data could be included in the motor analyses.

AIMS = Alberta Infant Motor Scale.

Figure 2. Flowchart representing the diagnostic value of each of the significant predictors for vestibular outcome, isolated and combined.

MRI = magnetic resonance imaging; NPV = negative predictive value; PPV = positive predictive value.

Figure 3. Motor outcome in patients with normal vestibular function compared to patients with vestibular dysfunction further subdivided according to the presence of the vestibular predictive variables (left panel), the laterality of the vestibular loss (middle panel) and the onset of the vestibular loss (right panel). Median values are displayed for each boxplot.

Z-scores $\leq$ -2,0 represent weak gross motor performance. * $0.05 < p < 0.01$; ** $0.01 < p \leq 0.001$ (Kruskal-Wallis test with Dunn's post hoc analyses with Bonferroni correction).

AIMS = Alberta Infant Motor Scale; PVC = periventricular cysts; SNHL = sensorineural hearing loss; VL = vestibular loss.

SUPPLEMENTARY INFORMATION LEGEND

Supplementary Table 1. Flemish classification of symptomatic cCMV-infection.