Vestibular Follow-up Program for Congenital Cytomegalovirus Based on 6 Years of Longitudinal Data Collection

Cleo Dhondt,[1] Leen Maes,[2,3] Sarie Martens,[2] Saartje Vanaudenaerde,[3] Lotte Rombaut,[3] Elise De Cuyper[1], Helen Van Hoecke,[1,3] Els De Leenheer,[1,3] and Ingeborg Dhooge [1,3]

[1]Department of Head and Skin, Ghent University, Ghent, Belgium; [2]Department of Rehabilitation Sciences, Ghent University, Ghent, Belgium; and [3]Department of Otorhinolaryngology, Ghent University Hospital, Ghent, Belgium.

Corresponding Author

Cleo Dhondt, Ghent University, Department of Head and Skin, Ghent, Belgium

E-mail: Cleo.Dhondt@UGent.be; Phone: 0032 9 332 28 89

Postal address: Ghent University Hospital, Department Head and Skin, Corneel Heymanslaan 10 (1P1), B – 9000 Ghent, Belgium

Conflicts of Interest and Source of Funding

None of the authors have conflicts of interest or financial relationships relevant to this article to disclose. No funding was secured for this study.

ABSTRACT

Objectives. Congenital cytomegalovirus (cCMV), the leading non-genetic cause of pediatric sensorineural hearing loss, can also affect vestibular function. Literature findings suggest clinical presentation of vestibular loss in cCMV to be as variable as the hearing loss. Still, probably due to the considerable additional burden it entails for both patients and diagnostic centers, longitudinal vestibular follow-up in cCMV is not well-established in clinical practice. Therefore, this study aims to propose an evidence-based vestibular follow-up program with proper balance between its feasibility and sensitivity.

Design. In this longitudinal cohort study, 185 cCMV-patients (mean age 3.2 years, SD 1.6 years, range 0.5 to 6.7 years) were included. Vestibular follow-up data were obtained through lateral video Head Impulse Test (vHIT) and cervical Vestibular Evoked Myogenic Potential (cVEMP) evaluations around the ages of 6 months, 1 year and 2 years. Around 3 and 4.5 years of age, data from vertical vHIT and ocular Vestibular Evoked Myogenic Potentials (oVEMP) were also collected.

Results. At birth, 55.1% (102/185) of patients were asymptomatic and 44.9% (83/185) were symptomatic. The mean duration of follow-up for all patients was 20.8 (SD 16.3) months (mean number of follow-up assessments: 3.2, SD 1.5). Vestibular loss occurred at some point during follow-up in 16.8% (31/185) of all patients. Six percent (10/164) of patients with normal vestibular function at first assessment developed delayed onset vestibular loss; 80.0% (8/10) of these within the first 2 years of life. Vestibular deterioration was reported both in patients who had been treated with postnatal antiviral therapy and untreated patients. At final evaluation, both the semicircular and the otolith system were impaired in the majority of vestibular-impaired ears (29/36, 80.6%). Dysfunctions limited to the semicircular system or the otolith system were reported in 4 (4/36, 11.1%) and 3 (3/36, 8.3%) ears, respectively. The occurrence

of vestibular loss was highest in patients with first trimester seroconversion (16/59, 27.1%) or with an unknown timing of seroconversion (13/71, 18.3%), patients with sensorineural hearing loss (16/31, 51.6%) and patients with periventricular cysts on magnetic resonance imaging (MRI) (7/11, 63.6%).

Conclusions. Longitudinal vestibular follow-up, most intensively during the first 2 years of life, is recommended in cCMV-patients with vestibular risk factors (first trimester or unknown timing of seroconversion; sensorineural hearing loss; periventricular cysts on MRI). If those risk factors can be ruled out, a single evaluation early in life (around 6 months of age) might be sufficient. Both semicircular and otolith system evaluation should be part of the follow-up program, as partial losses were reported.

INTRODUCTION

Congenital cytomegalovirus (cCMV), a herpes-like virus, affects 0.2 to 6.1% of all live births (Kenneson & Cannon 2007; Lanzieri et al. 2014). cCMV-infected infants are at risk for a wide range of neurodevelopmental disabilities, which can be present from birth or develop later in life (Leruez-Ville et al. 2020; Buca et al. 2021). Newborns with abnormalities on neonatal investigations are defined as symptomatic, but a universal definition is still lacking. The majority of a European expert panel stated that patients with central nervous system (CNS) involvement (e.g., convulsions, chorioretinitis, moderate to severe lesions on CNS imaging), severe single or multi-organ disease (e.g., hepatomegaly with liver failure) or isolated sensorineural hearing loss (SNHL) should be considered severely symptomatic. Patients with milder cCMV-related neonatal abnormalities are defined mildly or moderately symptomatic (Luck et al. 2017). Occurring in 10 to 32% of all cCMV-patients, SNHL is the most common sequela (Grosse et al. 2008; Goderis et al. 2014; Fletcher et al. 2018). The clinical presentation of cCMV-induced SNHL is typically very variable, as laterality, severity, onset and evolution remain largely unpredictable (Goderis et al. 2014; Fletcher et al. 2018; Vos et al. 2021). A pathophysiologic explanation for this unstable nature might be the coexistence of multiple pathogenic mechanisms. These include viral infection of a variety of cell types within the inner ear, with the stria vascularis and Reissner's membrane in the cochlea and dark cells in the vestibular apparatus suggested to be the primary targets, combined with host inflammatory responses (Teissier et al. 2011; Gabrielli et al. 2013; Teissier et al. 2016; Tsuprun et al. 2019; Moulden et al. 2021). These processes can disrupt not only the auditory, but also the vestibular function, as has been demonstrated by an increasing number of recent studies. Incidence rates for vestibular dysfunction in cCMV reported in literature cover a terribly wide range, from 14 to even 92% (Pappas 1983; Karltorp et al. 2014; Bernard et al. 2015; Dhondt et al. 2020; Pinninti et al. 2021; Shears et al. 2022), indicating the need for additional research in large,

representative study samples (Shears et al. 2022). The unpredictable nature of cCMV also holds true for vestibular outcome, as previous research has provided evidence for a wide range of vestibular dysfunctions, in terms of severity (uni- or bilateral, isolated parts of the vestibular organ or complete dysfunction of all 5 parts, mild function losses or complete areflexia), onset (present from the first examination onwards or delayed onset) and evolution (stable or progressive) (Pappas 1983; Huygen & Admiraal 1996; Zagólski 2008; Karltorp et al. 2014; Bernard et al. 2015; Maes et al. 2017; Dhondt et al. 2019b; Dhondt et al. 2020; Lazar et al. 2021; Pinninti et al. 2021; Chebib et al. 2022b). Although patients with SNHL seem to be most at risk, vestibular losses are also reported in asymptomatic and normal-hearing patients (Dhondt et al. 2020; Pinninti et al. 2021; Chebib et al. 2022b). Taken together, these findings endorse the need for longitudinal follow-up of the vestibular function in all cCMV-patients, as is already well-established for the auditory function (Goderis et al. 2014; Fletcher et al. 2018; Vos et al. 2021). However, evidence-based guidelines on how this should be carried out are still lacking (Corazzi et al. 2022; Shears et al. 2022). Besides, there might be reluctance to implement vestibular follow-up in the entire cCMV-population. On the one hand, this might be due the unfamiliarity with and the underestimation of the pivotal role of the vestibular system in various domains of development; early gross motor development being the most evident (Rine et al. 2000; Braswell & Rine 2006; Franco & Panhoca 2008; De Kegel et al. 2012; Lacroix et al. 2020; Singh et al. 2021). On the other hand, hesitancy might be related to feasibility issues from both diagnostic centers and patients and their families, as the burden of longitudinal follow-up may not be underestimated (Korndewal et al. 2018; Vandrevala et al. 2020; Shears et al. 2021). More specifically, this would be an addition to an already established wide-ranging cCMV-follow-up program, generally involving neurodevelopmental, ophthalmologic and auditory evaluation until primary school age (Luck et al. 2017). Therefore, a targeted vestibular evaluation program for cCMV could offer a solution to minimize the burden. A previous

analysis on a subset of our data identified hearing status, periventricular cysts on magnetic resonance imaging (MRI) and timing of seroconversion as important predictors for early vestibular outcome (manuscript accepted for publication). This is in line with recent findings of Chebib et al. (2022a) reporting antenatal imaging results and timing of seroconversion to be predictive of inner ear function (auditory and vestibular combined). Based on these previous findings and longitudinal data analysis in our large cohort of cCMV-patients, this study aimed to uncover the characteristics and natural course of vestibular function in cCMV to propose an evidence-based vestibular follow-up program with proper balance between its feasibility and sensitivity.

MATERIALS AND METHODS

Neonatal test battery in cCMV

There is no universal neonatal cCMV-screening in our country; children are tested for cCMV in case of cCMV-like symptoms (e.g., petechiae, SNHL) in the child, a known maternal seroconversion during pregnancy or in the context of neonatal screening offered in certain hospitals. Diagnosis of cCMV is established through virus isolation or CMV-DNA positive polymerase chain reaction (PCR) in a urine or saliva sample taken within the first 3 weeks of life (n=177) or retrospective analysis through CMV-DNA positive PCR on dried blood spot (Guthrie card, n=8). In our center, confirmed cCMV-diagnosis is followed by a series of neonatal tests including clinical examination, blood tests, cranial imaging (by means of ultrasound (US) and MRI), ophthalmologic evaluation and neonatal hearing evaluation. Abnormality on one of these examinations leads to the diagnosis of symptomatic cCMV, of which the severity (mild, moderate or severe) is further defined in accordance to the European consensus statement mentioned in the introduction (Luck et al. 2017; Keymeulen et al. 2021).

All other patients are defined asymptomatic. Patients identified through late retrospective cCMV-diagnosis because of delayed onset SNHL are, by definition, considered asymptomatic. With the exception of patients with isolated bilateral severe or profound SNHL, postnatal antiviral therapy is suggested in all patients with moderate or severe symptomatic cCMV. Detailed information on the neonatal test battery in patients suspected of cCMV can be found in Keymeulen et al. (2021).

Follow-up program

Children with a confirmed cCMV-diagnosis are enrolled in multidisciplinary follow-up. In our center, follow-up of cCMV-patients at the otorhinolaryngology department includes hearing and balance assessment, the latter involving both vestibular and motor examination.

Auditory testing. Neonatal hearing evaluation was carried out using automated auditory brainstem response (automated ABR). In case of refer, diagnostic ABR was performed within the first month after birth, combined with otomicroscopy and tympanometry to rule out otitis media. Further auditory follow-up, in which the regularity was depending on the cCMV (asymptomatic vs. symptomatic) and hearing (normal hearing vs. hearing-impaired) status, was carried out following a previously reported program (Goderis et al. 2016; Craeghs et al. 2020). This auditory follow-up program has been reduced from 6 to 4 years of age as from May 2019, following new scientific insights (Lanzieri et al. 2017; Foulon et al. 2019). Auditory data included in the current analyses involved click-evoked ABR thresholds, pure tone audiometry thresholds (average threshold at 500, 1000, 2000 and 4000 Hz) or, in case of normal-hearing ears without otitis media, transient evoked otoacoustic emissions. Middle ear pathology or conductive hearing loss was ruled out based on otomicroscopy, tympanometry and/or bone conduction thresholds. Neonatal ABR thresholds $\leq$ 40 dB nHL were considered normal. For follow-up measurements, click ABR-thresholds $\leq$ 30 dB nHL were classified as normal, between 31 and 45 dB nHL as mild, between 46 and 70 dB nHL as moderate, between 71 and

90 dB nHL as severe and ≥ 91 dB nHL as profound hearing loss (Madell & Flexer 2008). Pure tone average thresholds ≤ 25 dB nHL were defined as normal, between 26 and 40 dB HL as mild, between 41 and 70 dB HL as moderate, between 71 and 90 dB HL as severe, and ≥ 91 dB HL as profound hearing loss (International Bureau for Audiophonologie, recommendation 02/1 bis).

Patients' final auditory thresholds were included in the analyses, based on the hearing evaluation closest to the most recent vestibular examination (both measurements performed within one year of each other). Apart from that, onset and evolution of hearing loss were determined based on all auditory follow-up data available.

Vestibular end-organ testing. Standard longitudinal balance follow-up involved vestibular and motor assessment around the ages of 6 months, 1 year, 2 years, 3 years and 4.5 years. Further follow-up beyond that age was scheduled in case of diagnosis of vestibular loss or irregularities in earlier planning (e.g. due to cancellations by the parents or due to the covid pandemic). Intermediate evaluations were sometimes scheduled e.g., in case of evolving vestibular loss, scheduled cochlear implantation or parental concern. Motor data were not taken into account in the current analyses but are discussed in our other report (manuscript accepted for publication). Below the age of 3 years, vestibular assessment consisted of video Head Impulse Testing (vHIT) of lateral semicircular canals (SCC), rotatory testing and cervical Vestibular Evoked Myogenic Potentials (cVEMP) with bone conduction stimulus. From the age of 3 years, vHIT for vertical SCCs and ocular Vestibular Evoked Myogenic Potentials (oVEMP) with minishaker were added to the program. Caloric assessment was always performed with water and only included in the final evaluation around 4.5 years of age (Dhondt et al. 2019a; Dhondt et al. 2020). Data from rotatory and caloric testing were not included in the current analyses. The former because it does not allow ear-specific evaluation and reliability in the pediatric population is difficult to ascertain. The latter because it could only be carried

out successfully in a limited number of patients (n=15), which would not allow any meaningful conclusion (Dhondt et al. 2019a). All other patients older than 4.5 years (n=20) had persisting middle ear conditions (otitis media or transtympanic drains) that hampered reliable caloric assessment or refused to cooperate. As VEMP measurements were carried out with bone conducted vibration stimuli, all data included in the current analyses are ear-specific and free of any impact of the child's middle ear status, making it an ideal basis for reliable interpretation (Dhondt et al. 2019a).

vHIT testing for evaluation of the lateral and vertical semicircular canals was carried out using a device with stand-alone camera (vHIT Ulmer version III, Synapsys, Marseille, France). The child was placed on its parent's lap and stimulated to focus on an interesting visual target held by the examiner behind the camera. vHIT maneuvers with an amplitude of 10 to 20° and peak velocity of 100 to 200/°s and 150 to 250°/s for the vertical and lateral SCCs, respectively, were performed by the second examiner standing behind the parent. Vestibulo-ocular reflex gain values between 0.7 and 0.4 were considered a mild SCC deficit (Martens et al. 2022a), values below 0.4 were classified as severe SCC deficit (Dhondt et al. 2020).

cVEMP assessment for saccular function evaluation was performed with bone conduction stimuli (type B71W, RadioEar, Middelfart, Denmark). Children were placed in supine position on a sloping pillow with the head rotated towards an interesting visual stimulus presented at the side of the nontest ear. Linear 500 Hz tone burst stimuli (1-2-1 ms) were presented at the ipsilateral mastoid at 59 dB HL (129 dB force level (FL)) intensity and 5 Hz repetition rate. Electromyographic (EMG) activity was monitored visually and recorded with a commercial system (Bio-Logic Navigator-Pro platform, Mundelein, IL, USA and Neuro-Audio version 2010, Neurosoft, Ivanovo, Russia) using self-adhesive electrodes with the noninverting electrodes placed at the midpoint of the sternocleidomastoid (SCM) muscles, the inverting electrode 1 to 2 cm below the sternoclavicular junction, and the ground electrode on the

forehead. Electrode and inter-electrode impedances were accepted if below 5 and 2 kOhm, respectively. For all measurements, 2 trials with comparable SCM tension were selected to confirm waveform reproducibility and to determine the average rectified interpeak amplitude. Values below 0.3 (Bio-Logic) or 1.3 (Neuro-Audio) were considered a mild saccular deficit (Martens et al. 2022a). The absence of a reproducible cVEMP-waveform was seen as a severe saccular deficit (Dhondt et al. 2020).

oVEMP testing for assessment of utricular function was carried out with a minishaker (type 4810, amplifier model 2718, Brüel & Kjær, Nærum, Denmark Hearing) placed at the forehead (Fz). In supine position, children were asked to watch a video on a tablet held at a predetermined point behind the child to obtain an upward gaze angle of 30°. Blackman window 500 Hz tone burst stimuli (2-0-2 ms) with a 5 Hz stimulus repetition rate were presented at 140 dB FL intensity (Vanspauwen et al. 2017). EMG activity was recorded (Neuro-Audio version 2010, Neurosoft, Ivanovo, Russia) through self-adhesive electrodes with the noninverting electrodes symmetrically on both inferior oblique muscles just below the lateral eye canthi, the inverting electrodes next to both medial eye canthi and the ground electrode on the forehead (Fpz). The same acceptable impedance levels were applied as for cVEMP-measurements. Two trials were selected on each side to check waveform reproducibility and to calculate the average interpeak amplitude. Amplitudes below 10 µV and the absence of a reproducible waveform were considered a mild and severe utricular deficit, respectively (Martens et al. 2022a).

Criteria for mild and severe deficits were based on center-specific normative data (Martens et al. 2022a) and a previous report on an intermediate analysis of cCMV-data (Dhondt et al. 2020), supplemented with additional normative data obtained with the Bio-Logic system in 27 control subjects with a mean age of 14.5 months (SD 9.4 months). Hearing aids and/or cochlear implants were turned off during ipsilateral measurements to avoid excessive blinking (vHIT) or electrical interference (VEMPs). More information on adjustments of the standard vestibular

test battery for application in the pediatric population can be found in previous reports (Dhondt et al. 2019a; Dhondt et al. 2020; Dhondt et al. 2021; Martens et al. 2022a)

Defining onset and evolution. SNHL or vestibular loss were defined as delayed onset when it was detected after at least one evaluation of normal function. Change of function from one category to a more severe category, from unilateral to bilateral or (for vestibular loss) from a more limited number to a more extensive number of affected parts (e.g., isolated lateral SCC dysfunction evolved to a combined lateral SCC and saccular dysfunction) was considered progression. Similar evolution in the opposite direction was classified as improvement. Succession of progression and improvement within one patient was seen as fluctuation.

Subjects

From June 2016 until November 2021, 217 patients with a first vestibular assessment before 2 years of age were enrolled in this longitudinal vestibular follow-up study. The study was approved by the ethical committee of our hospital (2015/1441) and children's parents were asked for informed consent, which was refused in 7 cases. Five patients were excluded because of an additional risk factor for vestibular loss (toxoplasmosis, severe hyperbilirubinemia, mastoiditis, asphyxia) and 20 patients were excluded because a minimum of at least one reliable lateral vHIT and one reliable cVEMP measurement could not be obtained within the complete follow-up. Evidently, the latter exclusion criterion predominantly occurred in patients with limited follow-up duration. Cochlear implant patients were not excluded from the study as they involve an important subpopulation of the overall cCMV-population and earlier research indicates that the impact of pediatric cochlear implantation surgery in our center is limited (Dhondt et al. 2021). This left a total of 185 patients (90 boys, 95 girls) and 590 vestibular assessments to be included in the current analyses. Patients' mean age at the end of the data collection in November 2021 was 3.2 years (SD 1.6 years, range 0.5 to 6.7 years).

Statistical analysis

All analyses were performed with SPSS software (IBM, version 27.0, Armonk, NY). Descriptives and proportions were calculated both on subject and on ear level. Kaplan-Meier plots were used to display the cumulative survival of auditory and vestibular function in time, within subgroups of patients with an auditory and vestibular dysfunction, respectively. Concerning the latter, only patients with dysfunctions on lateral vHIT or cVEMP were taken into account, as abnormalities on vertical vHIT or oVEMP could only be diagnosed as from the age of 3 due to the setup of the vestibular follow-up program. As a consequence, timing of these diagnoses did not allow any useful conclusion on the natural course of the disease.

RESULTS

Subjects

Of the 185 patients included in this study, 102 (102/185, 55.1%) were asymptomatic and 83 (83/185, 44.9%) were symptomatic (see Figure 1 and Table, Supplemental Digital Content 1, which summarizes the results of cCMV-related neonatal examinations in our sample). Maternal seroconversion occurred in 59 patients (59/185, 31.9%) in the first trimester of pregnancy and in 38 (38/185, 20.5%) and 17 (17/185, 9.2%) in the second and third trimester, respectively. In all other patients (71/185, 38.4%), timing of seroconversion was unknown. Children's average age at first vestibular assessment was 7.2 (SD 3.0) months. With respect to the duration of the vestibular follow-up, eighteen patients were only followed until 6 months of age, 50, 42 and 40 until 1, 2 and 3 years, respectively, and 35 until 4.5 years or beyond. The mean duration of vestibular follow-up at the end of data collection was 20.8 (SD 16.3) months and patients had on average 3.2 vestibular follow-up assessments (SD 1.5).

Inner ear impairment

SNHL occurred in exactly the same number of patients at some point during follow-up as vestibular loss, i.e. in 31 (31/185, 16.8%) children. The auditory and the vestibular loss appeared to be resolved at final evaluation in 1 and 2 patients, respectively. Persisting SNHL and vestibular dysfunction occurred in 30 (30/185, 16.2%) and 29 (29/185, 15.7%) patients, respectively. Auditory and vestibular loss co-occurred in 15 patients (15/185, 8.1%), 15 patients (15/185, 8.1%) had isolated auditory impairment and 14 patients (14/185, 7.6%) had isolated vestibular impairment at last evaluation. Consequently, inner ear dysfunction (auditory and/or vestibular) at final evaluation occurred in 44 patients (44/185, 23.8%) in total (see Table, Supplemental Digital Content 2, which provides an overview of the patients with inner ear dysfunction during follow-up). Laterality and side of the auditory and vestibular impairment did not correspond in 34 (34/44, 77.3%) and 36 (36/44, 81.8%) subjects with inner ear impairment at final evaluation, respectively (Table 1). Comparison of onset, evolution and laterality of both inner ear impairments is illustrated in Figure 2.

SNHL was detected at birth in 21 (21/31, 67.7%) patients and with delayed onset in 10 (10/31, 32.3%) subjects. The latter involved 6.1% (10/164) of all patients born with normal hearing. After diagnosis, the severity of the auditory impairment remained stable in 18 (18/31, 58.1%) patients. Progressive, improving and fluctuating hearing loss were reported in 9 (9/31, 29.0%), 2 (2/31, 6.5%) and 1 (1/31, 3.2%) patients, respectively. In 1 (1/31, 3.2%) patient, the evolution of the delayed-onset hearing loss was unknown because SNHL had just been diagnosed at last evaluation before the end of data collection. At final evaluation, 18 (18/30, 60.0%) patients had unilateral and 12 (12/30, 40.0%) patients had bilateral SNHL. The degree of SNHL was profound in 30 of the 42 (71.4%) hearing-impaired ears. Only a minority of ears had mild (2/42, 4.8%), moderate (6/42, 14.3%) or severe (4/42, 9.5%) SNHL at final evaluation. Twenty hearing-impaired ears (20/42, 47.6%) received a cochlear implant.

Vestibular loss occurred at first assessment in 21 (21/31, 67.7%) patients and only after at least one normal evaluation in 10 (10/31, 32.3%) children (10/164, 6.1% of patients with normal vestibular function at first evaluation). The majority of patients had progressive (5/31, 16.1%), improving (6/31, 19.4%) or fluctuating (7/31, 22.6%) vestibular loss. Stable vestibular impairment was recorded in 10 patients (10/31, 32.3%). In the other 3 patients (3/31, 9.7%), diagnosis of vestibular impairment happened at the last included assessment so that evolution could not be determined. The natural course of vestibular function is demonstrated through longitudinal lateral vHIT and cVEMP data in patients with vestibular impairment, displayed in Figure 3. Twenty-two patients (22/29, 75.9%) had unilateral and 7 (7/29, 24.1%) had bilateral vestibular loss, resulting in 36 vestibular-impaired ears at last evaluation. Both the SCC and the otolith system were impaired in the majority of vestibular-impaired ears (29/36, 80.6%). Dysfunctions limited to the SCC system or the otolith system were determined in 4 (4/36, 11.1%) and 3 ears (3/36, 8.3%), respectively. The occurrence of dysfunction in each of the 5 parts of the vestibular organ is depicted in Figure 4. In the group of patients older than 3 years of age in which all 5 parts of the vestibular organ were evaluated, abnormalities on posterior vHIT were the most and abnormalities on cVEMP the least common. None of these patients had an isolated cVEMP abnormality without abnormality on one or more of the other tests. Eleven of the 36 vestibular-impaired ears (11/36, 30.6%) received a cochlear implant. Vestibular dysfunction was detected already before implantation in 9 (9/36, 25.0%) of them.

Age at diagnosis

The mean age at diagnosis of SNHL was 4.2 (SD 8.2) months, whereas 17.3 (SD 11.3) months was the mean age at diagnosis of vestibular loss. Kaplan-Meier curves illustrating the cumulative survival over time for auditory and vestibular function within the subgroups of patients with SNHL (n=31) and vestibular loss (only deficits on lateral vHIT and/or cVEMP, n=28) are plotted together in Figure 5. Note that the time window in which the first evaluation

of auditory function was scheduled (0-1 months) differs greatly from that in which the first evaluation of vestibular function was scheduled (6-24 months). As 19 patients (19/31, 61.3%) were already diagnosed with SNHL in the first month of life, and 5 patients (5/28, 17.9%) were already diagnosed with vestibular loss at 6 months of age, Kaplan Meier curves for SNHL and vestibular loss start at 38.7% and 82.1%, respectively. Out of 10 patients with confirmed delayed onset vestibular loss, 4 (4/10, 40.0%) patients were diagnosed around 1 years of age, 4 (4/10, 40.0%) patients around 2 years of age and 2 patients (2/10, 20.0%) thereafter (35 and 45 months, see Fig. 5).

Within the subgroup of 28 patients with abnormalities on lateral vHIT and/or cVEMP measurements, vHIT abnormality preceded an abnormal cVEMP-measurement in 2 patients (2/28, 7.1%). The reverse finding was reported in 1 patient (1/28, 3.6%). In 4 patients (4/28, 14.3%), the sequence of events was uncertain as both measurements were successful at a different time in follow-up (in 3 patients cVEMP could be carried out successfully before vHIT; in 1 patient it was the other way around). In 15 patients (15/28 53.6%), vHIT and cVEMP abnormality were reported at the same time. In the remaining 6 patients (6/28, 21.4%), the vestibular deficit (lateral vHIT and/or cVEMP) was limited to the SCC (n=5) or the otolith (n=1) system.

Risk factors

Vestibular loss occurred in 10.8% (11/102) of the asymptomatic and in 24.1% (20/83) of the symptomatic patients. The occurrence of vestibular loss (occurring during follow-up before 2 years of age, and before the end of complete follow-up) in relation to the timing of seroconversion, hearing status and the presence of periventricular cysts on MRI is illustrated in Table 2. This table shows that 16 patients with vestibular loss (16/31, 51.6%) had associated SNHL at some point during follow-up. In all but 2 patients (14/16, 87.5%), the diagnosis of SNHL preceded the diagnosis of vestibular loss with the time interval ranging from 3 to 45

months. In 1 patient (1/16, 6.3%), it was the other way around (time interval 5 months) and in another (1/16, 6.3%), vestibular and auditory loss were diagnosed at the same time. Highest occurrence of vestibular loss was reported in patients with first trimester or unknown timing of seroconversion, patients with co-occurring SNHL and patients with periventricular cysts on MRI. The occurrence of vestibular loss was as high as 22.6% (30/133) in patients having one of these risk factors compared to only 1.9% (1/52) when none of the risk factors occurred (Table 2).

DISCUSSION

As the clinical presentation of vestibular loss in cCMV is highly variable with potentially delayed onset or progressive losses occurring in both normal-hearing and hearing-impaired subjects, longitudinal follow-up assessment in a large and representative cCMV-cohort is the only appropriate approach to determine the optimal follow-up program in this population. This is the first study with adequate data to take up this challenge.

Characteristics of cCMV-induced vestibular loss

Up until today, the impact of cCMV on the vestibular system remains far less widely known than the auditory sequelae (Corazzi et al. 2022; Shears et al. 2022). However, our data show that the occurrence of both sequelae is identical, i.e., 17%. This occurrence rate is slightly higher than the 14% vestibular losses previously reported based on an intermediate analysis of data in 2019 (Dhondt et al. 2020). This is probably due to the longer follow-up duration (with a higher number of delayed onset dysfunctions) with more extensive vestibular evaluation (including vertical SCC and utricle) and the current additional inclusion of mild dysfunctions (whereas our previous report only included severe dysfunctions). Note that the current occurrence rate might still be a slight underestimation because isolated mid or low frequency losses of the lateral SCC

were not taken into account as data from rotatory and caloric testing were excluded from the analysis. However, this occurrence rate is considerably lower compared to the 45 to 92% reported in other centers (Pappas 1983; Karltorp et al. 2014; Bernard et al. 2015; Pinninti et al. 2021; Chebib et al. 2022a). This might be largely due to the fact that our proportion of symptomatic patients (45%) was considerably smaller compared to most previous studies in which the majority of patients were symptomatic (Pappas 1983; Karltorp et al. 2014; Bernard et al. 2015; Chebib et al. 2022a). Although there is still a slight overrepresentation of symptomatic patients in our sample, we believe our occurrence rate is the best approximation of the real incidence of vestibular loss in the cCMV-population, as our sample is the largest and most representative for the overall cCMV-population in which asymptomatic and normal-hearing patients are more common than symptomatic and hearing-impaired patients. Moreover, our vestibular occurrence rate is in accordance with the overall incidence rates for SNHL between 10 and 32% reported in literature (Grosse et al. 2008; Goderis et al. 2014; Fletcher et al. 2018), which could indeed be expected from the shared pathophysiology of auditory and vestibular damage in cCMV (Teissier et al. 2011; Gabrielli et al. 2013; Teissier et al. 2016; Tsuprun et al. 2019; Moulden et al. 2021).

The characteristics (onset, evolution, laterality) of both inner ear impairments show obvious similarities. Nevertheless, unilateral losses were slightly more common in vestibular compared to auditory impairments (Fig. 2). In fact, roughly 1 in 4 vestibular dysfunctions was bilateral. This is somewhat reassuring, as the motor outcome in bilateral losses is significantly worse than in unilateral dysfunctions (Wiener-Vacher et al. 2012; Inoue et al. 2013; Martens et al. 2022c). However, it should be noted not all 185 patients went through the complete 4.5 year-follow up, which includes an inherent underestimation of the reported results in this paper. With respect to the percentage of uni- or bilateral vestibular losses, the possibility that unilateral dysfunctions could still progress to bilateral losses should be kept in mind.

In the vast majority of patients, vestibular impairment was unstable in time (Fig. 2), underlining the need for longitudinal follow-up. Moreover, this could have important repercussions on a child's daily life. In fact, despite children's superior plasticity and central compensation abilities, it is plausible that altering vestibular function might hamper the efficacy of their central compensation strategies even more than steady function loss (Dutia 2010). In this respect, one might assume that deterioration is more inconvenient than improvement. Therefore, caregivers, parents and therapists should be aware that in 39% of our patients with vestibular dysfunction, deterioration was reported at some point (23% with fluctuation, 16% with progression). Interestingly, Figure 3 did not show an important protective effect of postnatal antiviral treatment on the vestibular function, as significant vestibular deteriorations were certainly not confined to untreated patients. However, more extensive research in a more standardized setting is necessary to make meaningful statements about this complicated issue (Shears et al. 2022).

Although the low proportion of bilateral vestibular losses is fortunate, it should be noted that dysfunctions were generally pronounced on the ear-level: both auditory and vestibular losses were more often severe than mild. This is in line with previous literature on auditory (Fowler & Boppana 2006; Goderis et al. 2014) and vestibular (Bernard et al. 2015) function losses in cCMV. As for the vestibular losses, both the SCC and the otolith system were affected in the majority of cases. However, as isolated SCC or otolith dysfunctions did occur, the use of one vestibular screening test does not seem sufficient to use as a starting point to recommend further extensive vestibular testing, which is a valuable approach for some other at risk populations (Martens et al. 2022b). The sequence of events supports this conclusion: vHIT abnormalities preceded cVEMP abnormalities in some patients, but in others, the reverse finding was reported. Also, Figure 4 demonstrates that the occurrence of vestibular loss had roughly the same magnitude for all 5 parts of the vestibular organ, with only a slightly higher proportion of

(posterior) vHIT abnormalities and a slightly lower proportion of cVEMP abnormalities. This finding, together with the fact that vHIT is a fast and child-friendly test that provides ear-specific information on 3 of the 5 parts of the vestibular organ, encourages a pivotal role of the vHIT in the vestibular follow-up of cCMV-patients. However, vHIT is slightly more difficult to carry out reliably in very young children, requires the use of adapted equipment (with stand-alone camera instead of goggles) and was less successful at a very young age than cVEMP-assessment (because of recording difficulties of the baby blue eyes by the infrared camera). Moreover, the clinical importance of the cVEMP as an evaluation of the otolith system should not be underestimated, as several studies have highlighted the greater impact of otolith dysfunctions on the early motor development compared to SCC losses (Abadie et al. 2000; Inoue et al. 2013). This altogether emphasizes the complementarity of vHIT and cVEMP to ensure an evaluation of both the SCC and the otolith system at all times.

Interestingly, in our subsample of patients with complete vestibular examination (beyond 3 years of age), no isolated cVEMP (saccule) abnormalities were recorded. Based on histopathological findings, it could be hypothesized that this could be related to the fact that the saccule is the only component in the vestibular organ without dark cells (Teissier et al. 2011; Gabrielli et al. 2013; Tsuprun et al. 2019). As a result, the virus, assuming that it enters the vestibular system through the dark cells, would have to intrude via one of the SCCs or the utricle before it could affect saccular function in a secondary stage.

Duration of vestibular follow-up

The mean age at diagnosis of SNHL (4.2 months) was considerably lower than the mean age at diagnosis of vestibular loss (17.3 months). This discrepancy is also visible in the Kaplan Meier plots (Fig. 5) and could be largely due to the setup of the auditory and vestibular follow-up program in our center. To the best of our knowledge, no center offers a more comprehensive vestibular follow-up for the entire cohort of cCMV-patients. Nevertheless, a significant time

difference remains between the first auditory and the first vestibular evaluation. Since the introduction of the Universal Neonatal Hearing Screening (UNHS), hearing evaluation before one month of age is provided for all children and as such also for all cCMV-infected children (Joint Committee on Infant Hearing 2000). In contrast, vestibular follow-up in our center is initiated around the age of 6 months. Besides, late cCMV-diagnosis after detection of delayed onset SNHL or referral from another center for extensive vestibular assessment led some subjects to enter the follow-up program later than 6 months so that the first evaluation was scheduled only many months later. This explains why vestibular diagnosis is made at a later age compared to the age at diagnosis of SNHL and implies that vestibular dysfunctions recorded "at first assessment" do not only include congenital, but also early onset (occurring before first assessment) dysfunctions. This means that the proportion of delayed onset vestibular dysfunctions (Fig. 2) is possibly still underestimated.

As 6% of all patients with normal vestibular function at first assessment developed delayed onset vestibular loss and 39% of all vestibular losses showed deterioration at some point, longitudinal follow-up is needed. Kaplan Meier plots from Figure 5 might aid in deciding on the duration and intensity of follow-up. It is striking that more than 90% of all auditory and vestibular dysfunctions and 80% of the delayed onset vestibular losses occurred within the first 2 years of life. The fact that the majority of delayed onset hearing losses occur before 2 years of age has already been reported (Goderis et al. 2016; Lanzieri et al. 2017; Cushing et al. 2022). Interestingly, this is the first study to indicate that this finding also holds true for the vestibular function. Again, it should be noted that our findings should be interpreted with caution as the number of patients that was followed beyond the age of 2 was smaller than the number of patients with shorter follow-up which could include a slight underestimation of the delayed onset dysfunctions beyond the age of 2.

Target populations

In a previous analysis, timing of seroconversion, hearing status and periventricular cysts on MRI emerged as important predictors for early vestibular outcome (manuscript accepted for publication). Timing of seroconversion and the development of SNHL are of course strongly related, as previously reported in literature (Chatzakis et al. 2020). However, both variables have a separate added value in predicting vestibular outcome as information on both is not always available at birth. Note that 52% of children with SNHL and even 75% of patients with bilateral SNHL had vestibular loss at last evaluation (Tables 1 and 2). This, and the fact that all but one bilateral vestibular loss occurred in hearing-impaired patients (Table 1), demonstrate the significant value of hearing status in predicting vestibular loss. Timing of seroconversion, on the other hand, has more of a negative predictive value. In fact, as vestibular loss occurred in only 5% of second trimester seroconversions and none of the third trimester seroconversions, these patients and their parents can be fairly reassured. Apart from these 2 predictors that are, to a greater or lesser extent, already described in literature (Zagólski 2008; Karltorp et al. 2014; Bernard et al. 2015; Chatzakis et al. 2020; Dhondt et al. 2020; Chebib et al. 2022a; Chebib et al. 2022b), the predictive value of periventricular cysts on MRI also looks promising. When available, these data could add to the accuracy to predict vestibular outcome.

With respect to hearing outcome, many studies report a higher incidence of SNHL in symptomatic patients compared to asymptomatic patients, frequently resulting in a different follow-up proposal for both groups (Grosse et al. 2008; Goderis et al. 2014; Vos et al. 2021). Although vestibular losses were more common in symptomatic patients (24%) compared to asymptomatic patients (11%), these results should be interpreted with caution. As in our center cCMV-patients with congenital SNHL are considered symptomatic, higher occurrence of vestibular loss in the symptomatic group is not surprising. More importantly, in our previous study where we controlled for this issue, severity of the cCMV-infection had no significant predictive value for vestibular outcome (manuscript accepted for publication). This is also in

line with the results of Chebib et al. (2022a), who reported the symptomatic status in cCMV to be a poor predictor for inner ear dysfunction.

Evidence-based vestibular follow-up proposal

The above findings added to the development of an evidence-based program as presented in Figure 6. The key premise is that longitudinal follow-up should be provided for patients with increased risk for vestibular dysfunction, i.e., patients with first trimester or unknown timing of seroconversion, patients with SNHL (congenital or delayed) and patients with periventricular cysts on MRI. If those risk factors can be ruled out, a single evaluation around the age of 6 months seems the right balance between sensitivity and feasibility issues.

In our sample, 23% (30/133) of the "at risk" patients developed vestibular loss. As the majority of vestibular losses was diagnosed within the first 2 years of life and the time frame from birth until independent walking seems to be the critical period for vestibular input to have a drastic impact on gross motor development (Wiener-Vacher et al. 2012), frequent follow-up up until the age of 2 seems appropriate. After the age of 2, a single additional, extensive vestibular evaluation around the age of 4 years (as is the suggested duration of auditory follow-up by Foulon et al. (2019) and Lanzieri et al. (2017)) might be sufficient. For patients that have been diagnosed with vestibular loss by the age of 2, an additional evaluation at the age of 3 does seem appropriate to ensure early detection of deterioration and therapy plans that are adjusted to patients' current vestibular status. Besides, at the age of 3, more comprehensive vestibular evaluation becomes feasible, which is of course extremely relevant in patients with already established vestibular dysfunction. Moreover, in these vestibular-impaired patients, further follow-up beyond the age of 4 might be recommended as late progression throughout the adolescence years are reported for hearing (Lanzieri et al. 2017; Demmler-Harrison & Miller 2020). Further research is needed to determine if this also holds true for the vestibular function.

The fact that we recommend a single early evaluation for the subgroup of patients without any of the risk factors, entails a drastic reduction of the burden for a significant part of the cCMV-population (in our sample 52/185, 28%) compared to when longitudinal follow-up would be applied for all cCMV-patients. However, in many regions with less comprehensive cCMV follow-up programs (e.g., without standard MRI evaluation or regions in which maternal cCMV screening is less common), important information on the described risk factors might be missing so that the proportion of patients that can be trusted to a single vestibular evaluation will be more limited. In our dataset, only 1 patient (1/52, 2%) within the subgroup of patients without risk factors developed (delayed onset, unilateral) vestibular loss. This is the only patient who would remain undetected with the proposed follow-up of Figure 6. However, as Chatzakis et al. (2020) reported that SNHL after second or third trimester seroconversion is generally less severe, we might expect the same for the vestibular function. Nevertheless, a single assessment remains appropriate because all cCMV-patients should still be considered at risk for vestibular loss. Just like a single vestibular assessment around the age of 6 months should be regarded as a minimum for patients with SNHL (Martens et al. 2019; Martens et al. 2022c), the same should apply for cCMV-patients. This single evaluation makes an incredible difference compared to no evaluation at all, because it enables to at least detect the congenital (or early onset) losses with the most detrimental impact on the early motor development (Wiener-Vacher et al. 2012) and it provides a reference measurement in case delayed onset abnormalities do occur. Also, this early vestibular testing holds a golden opportunity to increase awareness for the potential impact of cCMV on vestibular function and the associated functional implications. This could enable parents to recognize (potentially delayed onset) vestibular disorders that they would otherwise have been completely unaware of (Vandrevala et al. 2020).

In line with the longitudinal follow-up program based on scientific findings (Dhondt et al. 2019a; Martens et al. 2022a) and clinical experience we developed for this study, lateral vHIT

554 and cVEMP measurements seem the preferred combination to enable swift and ear-specific
555 information in a child-friendly manner, independent of the middle ear status during the first
556 years of life. As from the age of 3, when a child's cooperation has increased, vertical vHIT and
557 oVEMP should be added to the program.

558 Note that the proposal in Figure 6 should simply be seen as a guideline of which the frequency,
559 duration and content of vestibular follow-up should be adapted to every child's individual
560 needs. In children with persisting motor or vestibular complaints without abnormalities on the
561 applied vestibular assessment, for example, earlier and/or more extensive examinations should
562 be considered. Moreover, although Figure 6 only covers vestibular follow-up, diagnosis of
563 vestibular dysfunction should obviously urge to refer for functional (e.g., motor, visual acuity)
564 assessment to evaluate the impact on the child's daily function and discuss the need for therapy.
565 Although we aimed to take the feasibility of our follow-up proposal into account, the result
566 might still not be possible for many centers (Shears et al. 2021). In that case, it is important to
567 keep in mind that a single vestibular evaluation already entails a critical difference for a child's
568 developmental outcome and that the described risk factors can guide in the implementation of
569 a targeted approach (manuscript accepted for publication). Also, deployment of other available
570 equipment (e.g., rotatory test) or bedside screening tests (e.g., Head Impulse Test, postrotatory
571 nystagmus test) can be considered if the proposed instrumental test battery (Fig. 6) is not
572 attainable. Also, putting additional efforts into counselling and informing both parents and
573 caregivers might be equally important to facilitate early detection and rehabilitation of
574 vestibular dysfunctions in the cCMV-population.

575 Future research could investigate the added value of vestibular assessment even before the age
576 of 6 months, e.g., included in the multidisciplinary neonatal evaluation after cCMV-diagnosis.
577 However, the feasibility of such a neonatal vestibular screening has to be explored. For
578 example, objective evaluation of SCC function with vHIT is only possible from the age of 3

months (Wiener-Vacher & Wiener 2017). In contrast, saccular evaluation by means of cVEMP has been described to be feasible in neonates (Young et al. 2009) but vestibular dysfunction or severe hypotonia (commonly reported in cCMV) might complicate the buildup of sufficient muscle tension necessary for reliable interpretation. However, if demonstrated to be feasible and reliable, neonatal vestibular screening could enable vestibular dysfunction to be considered in the (a)symptomatic diagnosis of a patient, provide more insight in the true onset of vestibular deficits and enable parents to initiate motor therapy at an even earlier stage of development, as is already being done in preterm babies or infants with cerebral palsy (Spittle et al. 2015; Novak et al. 2017). Especially in cases with congenital vestibular areflexia, this could enable professional parental support and advice.

Apart from that, future research could further address the limitations of our current study and attempt to move towards an even better determination of the incidence of vestibular dysfunction, especially with respect to delayed onset cases; to get more insight in the impact of vestibular dysfunction on the motor outcome and to study the effect of antiviral therapy on long-term vestibular outcome. Future new insights could then give rise to further additions or refinements of the proposed program.

CONCLUSIONS

Vestibular loss in cCMV displays many similarities in incidence, onset, evolution, laterality, severity and predictive variables with auditory loss. However, as they are certainly not highly mutually correlated and can occur completely independently, predictions on inner ear impairments in cCMV remain extremely difficult. Anyway, it seems evident that vestibular assessment should be routinely implemented in clinical practice, in line with regular auditory follow-up that is already well-established. To ensure early detection of progressive or delayed

onset dysfunction, longitudinal follow-up is necessary, especially in patients with increased risk for vestibular dysfunction (with first trimester or unknown timing of seroconversion; SNHL; periventricular cysts on MRI) and with highest intensity during the first 2 years of life. If those risk factors can be ruled out, or if longitudinal follow-up is not possible, a single early evaluation (e.g., around the age of 6 months) might already make an incredible difference for a child's developmental outcome. Both semicircular and otolith system evaluation should be incorporated in the program, as partial losses were reported.

REFERENCES

Abadie, V., Wiener-Vacher, S., Morisseau-Durand, M. P., et al. (2000). Vestibular anomalies in CHARGE syndrome: investigations on and consequences for postural development. *Eur J Pediatr, 159,* 569-574.

Bernard, S., Wiener-Vacher, S., Van Den Abbeele, T., et al. (2015). Vestibular Disorders in Children With Congenital Cytomegalovirus Infection. *Pediatrics, 136,* e887-895.

Braswell, J., Rine, R. M. (2006). Evidence that vestibular hypofunction affects reading acuity in children. *Int J Pediatr Otorhinolaryngol, 70,* 1957-1965.

Buca, D., Di Mascio, D., Rizzo, G., et al. (2021). Outcome of fetuses with congenital cytomegalovirus infection and normal ultrasound at diagnosis: systematic review and meta-analysis. *Ultrasound Obstet Gynecol, 57,* 551-559.

Chatzakis, C., Ville, Y., Makrydimas, G., et al. (2020). Timing of primary maternal cytomegalovirus infection and rates of vertical transmission and fetal consequences. *Am J Obstet Gynecol, 223,* 870-883.e811.

Chebib, E., Maudoux, A., Benoit, C., et al. (2022a). Predictors of cochleovestibular dysfunction in children with congenital cytomegalovirus infection. *Eur J Pediatr*.

Chebib, E., Maudoux, A., Benoit, C., et al. (2022b). Audiovestibular Consequences of Congenital Cytomegalovirus Infection: Greater Vulnerability of the Vestibular Part of the Inner Ear. *Ear Hear*.

Corazzi, V., Hatzopoulos, S., Bianchini, C., et al. (2022). Vestibular and postural impairment in congenital Cytomegalovirus infection. *Int J Pediatr Otorhinolaryngol, 152*, 111005.

Craeghs, L., Goderis, J., Acke, F., et al. (2020). Congenital CMV-Associated Hearing Loss: Can Brain Imaging Predict Hearing Outcome? *Ear Hear, 42*, 373-380.

Cushing, S. L., Purcell, P. L., Papaiaonnou, V., et al. (2022). Hearing Instability in Children with Congenital Cytomegalovirus: Evidence and Neural Consequences. *Laryngoscope*.

De Kegel, A., Maes, L., Baetens, T., et al. (2012). The influence of a vestibular dysfunction on the motor development of hearing-impaired children. *Laryngoscope, 122*, 2837-2843.

Demmler-Harrison, G. J., Miller, J. A. (2020). Maternal cytomegalovirus immune status and hearing loss outcomes in congenital cytomegalovirus-infected offspring. *PLoS One, 15*, e0240172.

Dhondt, C., Dhooge, I., Maes, L. (2019a). Vestibular assessment in the pediatric population. *Laryngoscope, 129*, 490-493.

Dhondt, C., Maes, L., Oostra, A., et al. (2019b). Episodic Vestibular Symptoms in Children With a Congenital Cytomegalovirus Infection: A Case Series. *Otol Neurotol, 40*, e636-e642.

Dhondt, C., Maes, L., Rombaut, L., et al. (2020). Vestibular Function in Children With a Congenital Cytomegalovirus Infection: 3 Years of Follow-Up. *Ear Hear, 42*, 76-86.

Dhondt, C., Maes, L., Vanaudenaerde, S., et al. (2021). Changes in Vestibular Function Following Pediatric Cochlear Implantation: a Prospective Study. *Ear Hear*.

Dutia, M. B. (2010). Mechanisms of vestibular compensation: recent advances. *Curr Opin Otolaryngol Head Neck Surg, 18*, 420-424.

Fletcher, K. T., Horrell, E. M. W., Ayugi, J., et al. (2018). The Natural History and Rehabilitative Outcomes of Hearing Loss in Congenital Cytomegalovirus: A Systematic Review. *Otol Neurotol, 39*, 854-864.

Foulon, I., De Brucker, Y., Buyl, R., et al. (2019). Hearing Loss With Congenital Cytomegalovirus Infection. *Pediatrics, 144*.

Fowler, K. B., Boppana, S. B. (2006). Congenital cytomegalovirus (CMV) infection and hearing deficit. *J Clin Virol, 35*, 226-231.

Franco, E. S., Panhoca, I. (2008). Vestibular function in children underperforming at school. *Braz J Otorhinolaryngol, 74*, 815-825.

Gabrielli, L., Bonasoni, M. P., Santini, D., et al. (2013). Human fetal inner ear involvement in congenital cytomegalovirus infection. *Acta Neuropathol Commun, 1*, 63.

Goderis, J., De Leenheer, E., Smets, K., et al. (2014). Hearing loss and congenital CMV infection: a systematic review. *Pediatrics, 134*, 972-982.

Goderis, J., Keymeulen, A., Smets, K., et al. (2016). Hearing in Children with Congenital Cytomegalovirus Infection: Results of a Longitudinal Study. *J Pediatr, 172*, 110-115.e112.

Grosse, S. D., Ross, D. S., Dollard, S. C. (2008). Congenital cytomegalovirus (CMV) infection as a cause of permanent bilateral hearing loss: a quantitative assessment. *J Clin Virol, 41*, 57-62.

Huygen, P. L., Admiraal, R. J. (1996). Audiovestibular sequelae of congenital cytomegalovirus infection in 3 children presumably representing 3 symptomatically different types of delayed endolymphatic hydrops. *Int J Pediatr Otorhinolaryngol, 35*, 143-154.

Inoue, A., Iwasaki, S., Ushio, M., et al. (2013). Effect of vestibular dysfunction on the development of gross motor function in children with profound hearing loss. *Audiol Neurootol, 18*, 143-151.

Joint Committee on Infant Hearing. (2000). Year 2000 Position Statement: Principles and Guidelines for Early Hearing Detection and Intervention Programs. *Am J Audiol, 9*, 9-29.

Karltorp, E., Löfkvist, U., Lewensohn-Fuchs, I., et al. (2014). Impaired balance and neurodevelopmental disabilities among children with congenital cytomegalovirus infection. *Acta Paediatr, 103*, 1165-1173.

Kenneson, A., Cannon, M. J. (2007). Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Rev Med Virol, 17*, 253-276.

Keymeulen, A., Leenheer, D., Alexandra, C., et al. (2021). Results of a multicenter registry for congenital cytomegalovirus infection in Flanders, Belgium: From prenatal diagnosis over neonatal management to therapy. *Early Hum Dev, 163*, 105499.

Korndewal, M. J., Weltevrede, M., van den Akker-van Marle, M. E., et al. (2018). Healthcare costs attributable to congenital cytomegalovirus infection. *Arch Dis Child, 103*, 452-457.

Lacroix, E., Edwards, M. G., De Volder, A., et al. (2020). Neuropsychological profiles of children with vestibular loss. *J Vestib Res, 30*, 25-33.

Lanzieri, T. M., Chung, W., Flores, M., et al. (2017). Hearing Loss in Children With Asymptomatic Congenital Cytomegalovirus Infection. *Pediatrics, 139*.

Lanzieri, T. M., Dollard, S. C., Bialek, S. R., et al. (2014). Systematic review of the birth prevalence of congenital cytomegalovirus infection in developing countries. *Int J Infect Dis, 22*, 44-48.

Lazar, A., Löfkvist, U., Verrecchia, L., et al. (2021). Identical twins affected by congenital cytomegalovirus infections showed different audio-vestibular profiles. *Acta Paediatr, 110*, 30-35.

Leruez-Ville, M., Foulon, I., Pass, R., et al. (2020). Cytomegalovirus infection during pregnancy: state of the science. *Am J Obstet Gynecol, 223*, 330-349.

Luck, S. E., Wieringa, J. W., Blázquez-Gamero, D., et al. (2017). Congenital Cytomegalovirus: A European Expert Consensus Statement on Diagnosis and Management. *Pediatr Infect Dis J, 36*, 1205-1213.

Madell, J. R., Flexer, C. A. (2008). *Pediatric audiology: Diagnosis, technology, and management*. Thieme.

Maes, L., De Kegel, A., Van Waelvelde, H., et al. (2017). Comparison of the Motor Performance and Vestibular Function in Infants with a Congenital Cytomegalovirus Infection or a Connexin 26 Mutation: A Preliminary Study. *Ear Hear, 38*, e49-e56.

Martens, S., Dhooge, I., Dhondt, C., et al. (2019). Vestibular Infant Screening - Flanders: The implementation of a standard vestibular screening protocol for hearing-impaired children in Flanders. *Int J Pediatr Otorhinolaryngol, 120*, 196-201.

Martens, S., Dhooge, I., Dhondt, C., et al. (2022a). Pediatric Vestibular Assessment: Clinical Framework. Unpublished manuscript.

Martens, S., Dhooge, I., Dhondt, C., et al. (2022b). Three years of Vestibular Infant Screening in Infants with Sensorineural Hearing Loss. *Pediatrics*, Accepted for publication.

Martens, S., Maes, L., Dhondt, C., et al. (2022c). Vestibular Infant Screening - Flanders: What is the Most Appropriate Vestibular Screening Tool in Hearing-Impaired Children? *Unpublished manuscript*.

Moulden, J., Sung, C. Y. W., Brizic, I., et al. (2021). Murine Models of Central Nervous System Disease following Congenital Human Cytomegalovirus Infections. *Pathogens, 10*.

Novak, I., Morgan, C., Adde, L., et al. (2017). Early, Accurate Diagnosis and Early Intervention in Cerebral Palsy: Advances in Diagnosis and Treatment. *JAMA Pediatr, 171*, 897-907.

Pappas, D. G. (1983). Hearing impairments and vestibular abnormalities among children with subclinical cytomegalovirus. *Ann Otol Rhinol Laryngol, 92*, 552-557.

Pinninti, S., Christy, J., Almutairi, A., et al. (2021). Vestibular, Gaze, and Balance Disorders in Asymptomatic Congenital Cytomegalovirus Infection. *Pediatrics, 147*.

Rine, R. M., Cornwall, G., Gan, K., et al. (2000). Evidence of progressive delay of motor development in children with sensorineural hearing loss and concurrent vestibular dysfunction. *Percept Mot Skills, 90*, 1101-1112.

Shears, A., Fidler, K., Luck, S., et al. (2021). Routine Vestibular Function Assessment in Children With Congenital CMV: Are We Ready? *The Hearing Journal, 74*, 12,13,14.

Shears, A., Yan, G., Mortimer, H., et al. (2022). Vestibular and balance dysfunction in children with congenital CMV: a systematic review. *Arch Dis Child Fetal Neonatal Ed*.

Singh, A., Raynor, E. M., Lee, J. W., et al. (2021). Vestibular Dysfunction and Gross Motor Milestone Acquisition in Children With Hearing Loss: A Systematic Review. *Otolaryngol Head Neck Surg, 165*, 493-506.

Spittle, A., Orton, J., Anderson, P. J., et al. (2015). Early developmental intervention programmes provided post hospital discharge to prevent motor and cognitive impairment in preterm infants. *Cochrane Database Syst Rev, 2015*, Cd005495.

748 Teissier, N., Bernard, S., Quesnel, S., et al. (2016). Audiovestibular consequences of
749 congenital cytomegalovirus infection. *Eur Ann Otorhinolaryngol Head Neck Dis, 133*,
750 413-418.
751 Teissier, N., Delezoide, A. L., Mas, A. E., et al. (2011). Inner ear lesions in congenital
752 cytomegalovirus infection of human fetuses. *Acta Neuropathol, 122*, 763-774.
753 Tsuprun, V., Keskin, N., Schleiss, M. R., et al. (2019). Cytomegalovirus-induced pathology in
754 human temporal bones with congenital and acquired infection. *Am J Otolaryngol, 40*,
755 102270.
756 Vandrevala, T., Barber, V., Mbire-Chigumba, E., et al. (2020). Parenting a child with
757 congenital cytomegalovirus infection: a qualitative study. *BMJ Paediatr Open, 4*,
758 e000844.
759 Vanspauwen, R., Wuyts, F. L., Krijger, S., et al. (2017). Comparison of Different Electrode
760 Configurations for the oVEMP With Bone-Conducted Vibration. *Ear Hear, 38*, 205-
761 211.
762 Vos, B., Noll, D., Whittingham, J., et al. (2021). Cytomegalovirus-A Risk Factor for
763 Childhood Hearing Loss: A Systematic Review. *Ear Hear, 42*, 1447-1461.
764 Wiener-Vacher, S. R., Obeid, R., Abou-Elew, M. (2012). Vestibular impairment after
765 bacterial meningitis delays infant posturomotor development. *J Pediatr, 161*, 246-
766 251.e241.
767 Wiener-Vacher, S. R., Wiener, S. I. (2017). Video Head Impulse Tests with a Remote Camera
768 System: Normative Values of Semicircular Canal Vestibulo-Ocular Reflex Gain in
769 Infants and Children. *Front Neurol, 8*, 434.
770 Young, Y. H., Chen, C. N., Hsieh, W. S., et al. (2009). Development of vestibular evoked
771 myogenic potentials in early life. *Eur J Paediatr Neurol, 13*, 235-239.

772 Zagólski, O. (2008). Vestibular-evoked myogenic potentials and caloric stimulation in infants
773 with congenital cytomegalovirus infection. *J Laryngol Otol*, *122*, 574-579.

774

775

FIGURE LEGENDS

Figure 1. Overview of the (a)symptomatic status of the cCMV subject group and the proportion of patients that had antiviral treatment. Asympt, asymptomatic; mod, moderate; sympt, symptomatic.

Figure 2. Onset, evolution and laterality of auditory and vestibular impairment.

Figure 3. Evolution of vestibular function at ear-level in patients with vestibular loss on lateral vHIT (left panel) or cVEMP (right panel) in patients treated (solid lines) or untreated (dotdashed lines) with antiviral therapy. For cVEMP figures, only data obtained with Neuro-Audio equipment are presented. cVEMP, cervical Vestibular Evoked Myogenic Potentials; vHIT, video Head Impulse Test; VOR, vestibulo-ocular reflex.

Figure 4. Occurrence of dysfunction in each of the 5 parts of the vestibular organ. Ant, anterior semicircular canal; cVEMP, cervical Vestibular Evoked Myogenic Potentials; lat, lateral semicircular canal; oVEMP, ocular Vestibular Evoked Myogenic Potentials; post, posterior semicircular canal; vHIT, video Head Impulse Test.

Figure 5. Kaplan Meier plot representing the age at diagnosis for sensorineural hearing loss in hearing-impaired patients (dashed line; n=31) and vestibular loss in vestibular-impaired patients (solid line; n=28). Grey areas represent the time interval in which the first assessment was scheduled (0-1 months for hearing evaluation; 6-24 months for vestibular evaluation). Note that only patients with dysfunctions on the lateral video Head Impulse Test or cervical Vestibular Evoked Myogenic Potentials are included in the solid line of the Kaplan Meier analysis.

Figure 6. Proposed vestibular follow-up program for cCMV-patients. cCMV, congenital cytomegalovirus; cVEMP, cervical Vestibular Evoked Myogenic Potentials; FU, follow-up; lat, lateral semicircular canal; MRI, magnetic resonance imaging; oVEMP, ocular Vestibular

800 Evoked Myogenic Potentials; SNHL, sensorineural hearing loss; trim., trimester; vert, vertical
801 semicircular canals; vHIT, video Head Impulse Test.

SUPPLEMENTAL DIGITAL CONTENT

Supplemental Digital Content 1. Table that summarizes the results of cCMV-related neonatal examinations in our sample. pdf

Supplemental Digital Content 2. Table with an overview of the patients with inner ear dysfunction during follow-up. pdf