Congenital cytomegalovirus (CMV) infection affects 0.2–5% of all live-born neonates worldwide [1,2]. Only 10–15% of newborns with congenital CMV infection are symptomatic at birth [1]. Outcomes for symptomatic cases are poor with a mortality rate of 5%, and the majority of survivors may endure severe neurologic sequelae of which the major is sensorineural hearing loss [2,3]. Congenital CMV infection is the most common non-genetic cause of childhood congenital sensorineural hearing loss (up to 25%) [4]. In addition, 7–10% of the asymptomatic CMV infected babies may develop sequelae, including sensorineural hearing loss, which may only appear later at ages up to 6 years [1,5]. Therefore, it is important to detect all newborns with congenital CMV infection, including the asymptomatic cases, in order to establish a follow up plan for early detection of such sequelae.

Early studies with intravenous ganciclovir demonstrated improvement in hearing outcomes in symptomatic infants with central nervous system involvement [6]. Later, a 6-month course of oral valganciclovir was well-tolerated and effective therapeutic option for infants with symptomatic congenital CMV infection [7], thus defining this therapy as the standard of care.

Viral culture of urine and saliva are the gold standard for diagnosis of congenitally infected infants, although the current preferred method is the polymerase chain reaction (PCR) test for CMV in urine [8] and saliva [9] specimens, having sensitivity and specificity close to 100% and being more convenient than viral cultures.

In Israel, a two-stage universal newborn hearing screening program has been adopted in all hospitals since 2010. All neonates are tested in the first 3 days of life by transient evoked otoacoustic emissions (TEOAE), and those who fail undergo a second stage screening using automated auditory brainstem response (A-ABR). Neonates who do not pass automated auditory brainstem response testing in one or both ears, are referred for full diagnostic evaluation by brainstem evoked response audiometry (BERA) during the first 3 months of life. Asymptomatic newborns who fail neonatal auditory evaluation may constitute a high-risk group suitable for targeted CMV examination.
Until a cost-effective universal screening is adopted, one of the current strategies proposed for the detection of symptomatic newborns with congenital CMV infection is targeted examination, which aims to detect all newborns with congenital CMV infection who may be eligible for antiviral treatment. Through targeted examination, only infants with clinical features suggestive of CMV infection and/or newborns who fail to pass the routine newborn hearing screening tests are screened for CMV infection. According to this approach, virtually all newborns who would be candidates for antiviral treatment could be detected early, possibly leading to improved outcomes.

Thus, the aim of the study was to test the yield of a targeted examination for detecting infants with symptomatic congenital CMV infection in whom antiviral therapy is indicated, based on detection of the infection in newborns who failed to pass the newborn hearing screening tests, in addition to newborns with clinical features suggesting CMV.

PATIENTS AND METHODS

STUDY POPULATION

We conducted a prospective observational study of a cohort of patients between 1 January 2014 and 31 December 2015 at two medical centers in northern Israel: Emek Medical Center in Afula, and Ziv Medical Center in Safed.

The study included all newborns who either failed the first two stages of the routine hearing screening tests in one or both ears, and/or who were suspected to have congenital CMV infection according to clinical, laboratory or radiologic features, and/or newborns with suspected congenital CMV infection detected during pregnancy in mothers who had primary CMV infection.

The study received the approval of the ethics committee of both medical centers.

CONGENITAL CMV INFECTION DIAGNOSIS

A urine or saliva specimen was obtained from each included newborn before discharge from the hospital for detection of congenital CMV infection. Positive CMV PCR in saliva was confirmed by a urine specimen due to possible false positive results in breastfed newborns due to contamination by breast milk.

APPROACH TO NEWBORNS DIAGNOSED WITH CONGENITAL CMV INFECTION

Each newborn diagnosed with congenital CMV infection underwent the following workup (if not done before):

- Laboratory tests included complete blood count, chemistry for renal and hepatic function tests
- Cranial ultrasound for the detection of characteristic congenital CMV infection findings
- Ophthalmologic examination for the detection of chorioretinitis
- BERA before the age of one month

- Lumbar puncture for a PCR test for CMV in the cerebrospinal fluid if CNS infection was suspected based on clinical symptoms and signs.

All symptomatic newborns diagnosed with congenital CMV infection were treated with valganciclovir (with a dose of 16 mg/kg twice daily) for 6 months. In 2014, therapy was offered only to newborns with evidence of CNS involvement, for an overall of 6 months [10]. Since 2015, the treatment was offered to any newborn considered having a symptomatic CMV infection [7].

PCR TESTS

The tests were conducted in the molecular biology laboratory at the Emek Medical Center. DNA extraction from the urine or saliva specimen was conducted using the Easymag device, DNA amplification of specific target sites of CMV virus DNA based on real time PCR was conducted using the Rotor Gene Q device (Qiagen company) and RealStar® CMV QPC kit [11].

STATISTICAL ANALYSIS

In a study conducted in Israel, the incidence of congenital CMV infection in newborns was found to be 0.7% (0.5–1%), and one case (9%) was symptomatic [12]. The incidence of symptomatic congenital CMV treated infants in our study was compared with the expected rate in Israel and tested as the ratio of a Poisson variable to its expected value [13]. In a pilot study conducted in the Emek Medical Center 2.5% of newborns who failed the routine hearing screening were found to have a congenital CMV infection. According to these data, a total of 365 newborns who failed the hearing screening would be needed to achieve a statistical power of 80%, $P < 0.05$ was considered significant.

RESULTS

During the study period, 15,433 infants were born in the two centers. Overall 539 newborns (3.5%) failed to pass the first two steps of the hearing screening tests, of whom 5 (1%) had a positive urine PCR test for CMV; 3 at the Emek Medical Center and 2 at the Ziv Medical Center. Two newborns (40%) failed BERA and were treated with oral valganciclovir, while 3 newborns (60%) who did not fail BERA and did not have other stigmata of congenital CMV infection were followed clinically and by repeated hearing and developmental assessments. We tested 153 newborns (1%) for possible congenital CMV infection due to clinical, radiological, and laboratory abnormalities suggesting CMV infection. Of these infants 56 were tested due to primary CMV infection in whom antiviral therapy is indicated, based on detection of the infection in newborns who failed to pass the routine newborn hearing screening tests, of whom 5 (1%) had a positive urine PCR test for CMV; 3 at the Emek Medical Center and 2 at Ziv Medical Center.

Nine newborns (1.6%) had positive saliva CMV DNA PCR testing with concomitant negative urine CMV DNA PCR testing. These were considered as false positive results and were not included as infants with CMV infection.
Among the 13 symptomatic cases, 2 newborns (15%) received treatment with oral valganciclovir due to hearing impairment according to BERA, while 6 newborns (46%) received treatment for other reasons: 2 newborns were treated due to thrombocytopenia, one due to CMV pneumonitis, one due to small for gestational age (SGA) status, and two due to maternal CMV infection, as explained in the previous section.

Table 2 summarizes the 18 cases (of which 15 were symptomatic) who tested positive for CMV, both through the routine hearing screening and clinical features suggesting CMV infection and maternal CMV infection.

With regard to hearing impairment, 4 of the 15 symptomatic infants (25%) were diagnosed with hearing impairment by BERA testing; 2 of those were detected through the routine hearing screening tests, and 2 were detected by clinical findings (one due to thrombocytopenia and the other due to maternal CMV infection).

The symptomatic cases represented 0.1% of all newborns born at both medical centers during the study period. In Israel the incidence of congenital CMV infection (symptomatic and asymptomatic) is 0.7% [12]. Therefore, the estimated number of all newborns with congenital CMV infection in our study period would be 108, 11–16 of which would be expected to have symptomatic infection (10–15%).

The incidence of symptomatic congenital CMV infection during the current study period was compared with the expected rate in Israel. With 15 observed cases, the incidence ratio is 0.11 with 95% confidence interval: 0.066–0.175, reflecting no significant difference between the observed and expected cases (the interval includes unity). The data suggest that the targeted examination of only 692 (4.5%) newborns in our two study centers in Israel identified all the newborns with symptomatic congenital CMV infection in whom therapy was indicated.

DISCUSSION

In this study, we presented the yield of a targeted examination for detecting infants with symptomatic congenital CMV infection in whom antiviral therapy is indicated. The results show that the incidence of CMV treated infants in our study was similar to the expected rate in Israel. With 15 observed cases, the incidence ratio is 0.11 with 95% confidence interval: 0.066–0.175, reflecting no significant difference between the observed and expected cases (the interval includes unity). The data suggest that the targeted examination of only 692 (4.5%) newborns in our two study centers in Israel identified all the newborns with symptomatic congenital CMV infection in whom therapy was indicated.

MATERIAL PRIMARY CMV INFECTION CASES

During the study period, 56 newborns were tested due to primary CMV infection of the mother during pregnancy. Primary infection was suspected mainly due to symptoms of viral disease during pregnancy. Notably, a study in Israel found that 1% of women who underwent CMV screening during pregnancy tested positive [14]. The mean gestational age of testing for CMV infection (IgM) was 16.7 ± 8.7 weeks of gestation. Fourteen women underwent amniocentesis (mean gestational age 24.5 ± 5.1 weeks) and none tested positive for CMV PCR.

During pregnancy, one case was followed due to intra-uterine growth retardation (IUGR). In four cases there were abnormal findings in prenatal ultrasound (periventricular calcifications, ventriculomegaly and cystic lesions in the choroid plexus), and two cases with polyhydramnios.

In this group, average week of delivery was 38 ± 1 weeks, mean birth weight was 3182 ± 498 grams, and mean head circumference at birth was 34 ± 1.3 cm. None of the newborns had signs of congenital CMV infection on physical examination. Five infants did not pass the routine hearing screening tests, but only one had hearing impairment according to BERA testing.

Six newborns tested positive for CMV PCR after birth. Two of these newborns received treatment with oral valganciclovir: one due to hearing impairment according to BERA, and one due to evidence of periventricular calcifications on brain ultrasound.

Table 1. Clinical, radiological, and laboratory abnormalities in newborns in infants in whom congenital CMV infection was suspected on clinical grounds

<table>
<thead>
<tr>
<th>Cause for CMV testing</th>
<th>CMV PCR result</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>Maternal CMV infection</td>
<td>6</td>
<td>48</td>
<td>56</td>
</tr>
<tr>
<td>Intrauterine growth restriction</td>
<td>2</td>
<td>53</td>
<td>55</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>1</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Dysmorphic features</td>
<td>–</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Prolonged jaundice and elevated liver enzymes</td>
<td>–</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Liver calcification in ultrasonography</td>
<td>–</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Abnormalities in fetal brain ultrasonography (periventricular calcifications)</td>
<td>–</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Abnormalities in fetal abdominal ultrasonography (cysts)</td>
<td>–</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>140</td>
<td>153</td>
</tr>
</tbody>
</table>

CMV = cytomegalovirus, PCR = polymerase chain reaction

SYMPTOMATIC CMV CASES

Among the 13 symptomatic cases, 2 newborns (15%) received treatment with oral valganciclovir due to hearing impairment according to BERA, while 6 newborns (46%) received treatment for other reasons: 2 newborns were treated due to thrombocytopenia, one due to CMV pneumonitis, one due to small for gestational age (SGA) status, and two due to maternal CMV infection, as explained in the previous section.

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The incidence of symptomatic congenital CMV infection during the current study period was compared with the expected rate in Israel. With 15 observed cases, the incidence ratio is 0.11 with 95% confidence interval: 0.066–0.175, reflecting no significant difference between the observed and expected cases (the interval includes unity). The data suggest that the targeted examination of only 692 (4.5%) newborns in our two study centers in Israel identified all the newborns with symptomatic congenital CMV infection in whom therapy was indicated.

DISCUSSION

In this study, we presented the yield of a targeted examination for detecting infants with symptomatic congenital CMV infection in whom antiviral therapy is indicated. The results show that the incidence of CMV treated infants in our study was similar to the expected rate in Israel. With 15 observed cases, the incidence ratio is 0.11 with 95% confidence interval: 0.066–0.175, reflecting no significant difference between the observed and expected cases (the interval includes unity). The data suggest that the targeted examination of only 692 (4.5%) newborns in our two study centers in Israel identified all the newborns with symptomatic congenital CMV infection in whom therapy was indicated.

Nine newborns in our study had false positive salivary PCR CMV results, which emphasizes the need for caution in the use of saliva specimens and the need for confirmatory urine PCR CMV testing.
During the study period, 15 newborns were diagnosed with symptomatic CMV infection; 13 due to clinical features and 2 due to abnormal routine hearing screening. According to the literature and the number of live births in the years 2014–2015, it was estimated that 108 newborns would have congenital CMV infection, 16 of whom with symptomatic CMV infection. Our study results matched this estimation. This result carries great clinical significance because it strongly suggests that the targeted examination approach presented, based on clinical features suggesting CMV infection and on routine hearing screening, can detect virtually all newborns with symptomatic congenital CMV infection who might benefit from anti-viral treatment. Early identification and subsequent treatment of the babies could help to mitigate hearing loss and improve cognitive skills and language development. The beneficial effects of anti-viral treatment have only been shown when initiated during the first month of life, therefore early detection, as seen with this targeted examination approach, is of critical importance.

International consensus recommendations for congenital CMV diagnosis and management were published 2017, after collection of data in our study was completed. The group recommended that infants with asymptomatic congenital CMV infection should not receive antiviral treatment. Moreover, neonates with mild symptomatic disease or with isolated sensorineural hearing loss and no other disease manifestations should not routinely receive anti-viral treatment because of a lack of data suggesting benefit in this less severely affected population, and treatment is delivered on a case-by-case basis [16]. Trials are currently being conducted to examine whether early antiviral treatment of newborns with congenital cytomegalovirus infection and sensorineural hearing loss can prevent progression of hearing loss. Once results of these trials become available, our targeted examination can help detect those infants eligible for antiviral treatment.

In recent years, there has been an increased interest in adopting a targeted examination for the detection of congenital CMV infection [17]. One of the drawbacks of such a screening is that it misses newborns with asymptomatic congenital CMV infection who pass hearing screening. Of the 108 estimated cases of congenital CMV expected in both our medical centers in 2014–15, we detected 18 cases. An estimated 90 newborns with asymptomatic congenital CMV infection went undetected by our targeted examination approach. These asymptomatic new-

### Table 2. Positive CMV cases tested through the routine hearing screening tests and clinical features suggesting CMV infection

<table>
<thead>
<tr>
<th>Medical center</th>
<th>Reason for testing for CMV</th>
<th>Hearing impairment</th>
<th>Physical examination, lab tests or imaging findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emek, Afula</td>
<td>Failure in routine hearing screening tests</td>
<td>BERA hearing threshold 75 dB in both ears</td>
<td>–</td>
</tr>
<tr>
<td>Emek, Afula</td>
<td>Failure in routine hearing screening tests</td>
<td>BERA hearing threshold, Right ear 45 dB, Left ear 50 dB</td>
<td>–</td>
</tr>
<tr>
<td>Emek, Afula</td>
<td>Failure in routine hearing screening tests</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Emek, Afula</td>
<td>Maternal CMV</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Emek, Afula</td>
<td>Maternal CMV</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Emek, Afula</td>
<td>Thrombocytopenia, Hepatosplenomegaly</td>
<td>BERA hearing threshold, Right ear 30 dB, Left ear 55 dB</td>
<td>Thrombocytopenia, cholestatic jaundice</td>
</tr>
<tr>
<td>Emek, Afula</td>
<td>Thrombocytopenia</td>
<td>–</td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Emek, Afula</td>
<td>CMV Pneumonitis</td>
<td>Hearing impairment according to BERA (no detailed information available)</td>
<td>–</td>
</tr>
<tr>
<td>Ziv, Tsfat</td>
<td>Maternal CMV</td>
<td>–</td>
<td>SGA</td>
</tr>
<tr>
<td>Ziv, Tsfat</td>
<td>Symmetric SGA</td>
<td>–</td>
<td>SGA</td>
</tr>
<tr>
<td>Ziv, Tsfat</td>
<td>Maternal CMV</td>
<td>–</td>
<td>Periventricular calcifications in brain US</td>
</tr>
<tr>
<td>Emek, Afula</td>
<td>Microcephaly, symmetric SGA</td>
<td>–</td>
<td>SGA</td>
</tr>
</tbody>
</table>

BERA = brain stem evoked response, CMV = cytomegalovirus, SGA = small for gestational age
borns may develop sensorineural hearing impairment in their first years of life [1,5]. Similar results were found in a study by Fowler et al. [18].

Interestingly, a recent study suggested that infants with asymptomatic congenital CMV infection who were identified through newborn screening with normal hearing by age 2 years do not appear to have differences in IQ, vocabulary, or academic achievement scores during childhood or adolescence compared with uninfected children, implying that children with asymptomatic congenital CMV infection at birth may not need long-term monitoring for cognitive impairment or disabilities [19].

Universal systematic screening of newborns for congenital CMV infection has not been adopted yet mainly due to the lack of definitive studies on the cost-effectiveness of the various screening methods, although reliable data exists on the disease burden due to congenital CMV infection on the health system, including the various hearing loss treatments (e.g., hearing aids, cochlear implants, special education classes). A previous cost-benefit analysis reported a net public benefit for targeted CMV testing of neonates with hearing loss [15]. Additional cost-effectiveness studies are needed for recommending a universal cytomegalovirus testing of neonates. Until a universal screening for the detection CMV is adopted, which will allow the detection of all asymptomatic cases, we believe that the targeted examination we propose is a viable option for the detection of symptomatic newborns in whom valganciclovir therapy might be considered.

To the best of our knowledge, this is the first study that presents a targeted examination, which includes newborns with suspected CMV infection due to primary CMV infection of the mother during pregnancy, a policy that increases the yield of the targeted examination, especially by early diagnosis of asymptomatic cases that might develop hearing loss later in life.

CONCLUSIONS

Targeted examination of newborns with features suggesting CMV infection and newborns who failed to pass the routine newborn hearing screening effectively detected the estimated cases of symptomatic congenital CMV infection during the study period. This finding suggests that the examination policy can detect nearly all newborns with congenital CMV infection in whom therapy is indicated.

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