Basic science developments and opportunities for translational research

Interleukin-10 expression by cytomegalovirus-specific CD4+ T cells promotes virus persistence and is driven by type I IFN-induced interleukin-27 (ID 070)

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Abstract

Mucosal secretions represent a major source of infectious human cytomegalovirus (HCMV) in congenital infection. CD4+ T cells support mucosal host defense against herpesviruses and other viral pathogens. We identified that CD4+ T cells from systemic and mucosal tissues of hosts infected with β-herpesviridae human cytomegalovirus (HCMV) or murine cytomegalovirus (MCMV) express the regulatory cytokine interleukin (IL)-10. Mice lacking CD4+ cell-derived IL-10 elicited enhanced antiviral T cell responses and restricted MCMV persistence in salivary glands and secretion in saliva. Thus, IL-10+CD4+ T cells suppress antiviral immune responses against CMV. Expansion of this T-cell population in the periphery was strictly dependent on IL-27, and infected Il27r(alpha)-deficient mice displayed robust T cell responses and restricted MCMV persistence and shedding. Thus, IL-27 control of IL-10+CD4+ T cells promotes CMV transmission and persistence. IL-27 production was promoted by type I IFN, and our data suggest that β-herpesviridae exploit the immune-regulatory properties of this antiviral pathway to establish chronicity and enable horizontal transmission.

Basic science developments and opportunities for translational research

HUMAN CYTOMEGALOVIRUS PHOSPHOPROTEINS ARE HYPOPHOSPHORYLATED AND INTRINSICALLY DISORDERED (ID 080)

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Abstract

Brief introduction: Phosphorylation of proteins has important regulatory functions in cell homeostasis and is tightly regulated by counteracting forces of kinases and phosphatases. The tegument of human cytomegalovirus (CMV) is comprised of several proteins that are considered to be extensively phosphorylated but also of two cellular protein phosphatases (PP1 & PP2A).

Case report: To investigate this apparent conflict, we evaluated the phosphorylation status of the tegument proteins pUL32 and pp65 by enzymatic dephosphorylation and mass spectrometry. Enzymatic dephosphorylation with bacterial λ phosphatase, but not with PP1, shifted the pUL32-specific signal on reducing SDS-PAGE from 150 kDa to 148 kDa – still much larger than the ~118kDa obtained from our diffusion studies and from the calculated protein mass of ~113 kDa. Remarkably, inhibition of phosphates by treatment with the phosphatase inhibitors Calyculin A and Okadaic acid resulted in a shift to ~200 kDa or ~180, respectively, indicating that a considerable amount of potential phosphosites on pUL32 are not phosphorylated under normal conditions. Mass

spectrometry revealed a general state of hypophosphorylation of CMV phosphoproteins with only 17 phosphosites detected on pUL32 and 19 on pp65, respectively. Additional phosphoresidues were identified on envelope, transmembrane, tegument, and non-structural CMV proteins. Moreover, we found in silico evidence that the C-terminal two-thirds of pUL32 are intrinsically disordered and that most phosphorylations mapped to this region.

Conclusion: In this study, we show that important CMV tegument proteins are indeed phosphorylated though to a lesser extent than previously expected and the difference in migration velocity and calculated mass of pUL32 may not be attributed to phosphorylation but more likely be due to the partially intrinsically disordered nature of pUL32.

Basic science developments and opportunities for translational research

Impact of HCMV and antivirals on cytokine dysregulation in placental ex vivo model: a comparison between 1st and 3rd trimester. (ID 092)

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Abstract

Introduction: Human cytomegalovirus (HCMV) is the most common infectious cause of severe fetal malformation in developed countries. Using placental ex vivo models allow a better understanding of infection mechanism during pregnancy. We assessed placental damage with placental cytokine assays and tested impact of antiviral on viral infection and cytokine expression.

Materials and methods: Placental explants were infected with AD169 strain during 5 days before antiviral drug administration. Ganciclovir (GCV), foscarnet (FOS) and maribavir (MBV) were tested at 0.1xIC50, 1xIC50 and 10xIC50 concentrations (IC50 based in our previous studies). CMV and mock infected explants were harvested at days 4, 7, 10 and 13 dpi for analysis of levels of TNF-α, MCP-1 and IP-10 mRNA. Supernatants were analyzed for cytokine secretion using customized Bioplex assays.

Results: Tested antivirals inhibit more than 80% viral load in first trimester explants. In 3rd trimester explants, low concentrations of GCV and MBV show weaker inhibition. Preliminary results on cytokines mRNA show stronger pro-inflammatory cytokines expression in 3rd trimester whereas in 1st trimester, only TNF-α increases. In supernatants we observed more cytokines excreted in non-infected 1st trimester explants. AD169 infection increases IP-10 and TNF-α in 1st trimester supernatants and only IP-10 increases in 3rd trimester supernatants. Antivirals don't change cytokines expression in 1st and 3rd placenta tissue.

Conclusions: Our explant model shows differences of antiviral efficacy in 1st and 3rd trimester placenta. Increasing of pro-inflammatory cytokine expression may be correlated to fetus damages. Using ex vivo model is important to understand impact of viral infection in placenta.

Basic science developments and opportunities for translational research

HUMAN CYTOMEGALOVIRUS INDUCES A ROBUST INNATE IMMUNE RESPONSE IN THE MATERNAL-FETAL INTERFACE WITH DUAL ROLES IN ANTIVIRAL DEFENSE AND PLACENTAL DAMAGE (ID 105)

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Abstract

The earliest stages of human cytomegalovirus (HCMV) maternal-fetal transmission occur in the maternal decidua - representing the maternal aspect of the chimeric human placenta. The initial interplay between HCMV and the innate tissue response in the human maternal-fetal interface, though crucial for determining the outcome of congenital infection, has remained unknown in the absence of animal models for congenital HCMV. We have recently established an ex vivo model of HCMV infection and spread in human decidual tissues, maintained as multi-cell-type organ cultures.

Here we employed the decidual infection model to study the innate response to HCMV infection within the authentic milieu of the human decidua. Using both laboratory-derived and clinical HCMV strains from congenital HCMV cases, we showed that HCMV infection triggered a rapid and robust decidual-tissue innate immune response, predominated by interferon (IFN)γ and IP-10 induction, dysregulating the immune-tolerant decidual tissue cytokine/chemokine environment in a distinctive fashion. The decidual-tissue response was already elicited during viral-tissue binding, and was not affected by HCMV hyper-immune globulins. Of note, IFNγ induction, reflecting immune-cell activation, was distinctive to the maternal decidua, and was not observed in concomitantly-infected placental (fetal) villi. Importantly, functional analysis revealed that innate response mediators recovered from HCMV-infected decidual tissues exerted both antiviral protection and placental-damage activities.

Our studies in a clinically-relevant surrogate human model, provide a novel insight into the first-line decidual tissue response which could mediate the outcome of congenital HCMV infection.

CMV in Europe

HUMAN CYTOMEGALOVIRUS CONGENITAL INFECTION: IMPLEMENTATION OF A SCREENING PROGRAM DURING PREGNANCY (ID 065)

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Abstract

Introduction:

Although congenital CMV is the leading cause of congenital infection in our population, serological screening during pregnancy is still controversial. Many newborns will be asymptomatic at birth but will be at risk of developing long term neurological sequelaes during childhood. Adequate knowledge of the infection prevalence and its consequences in our population might lead to a better treatment and follow up of this patients.

Case report:

Matherial and Methods:

From January 2010 until 2014 all pregnant woman at Quiron Madrid Hospital underwent systematic serological screening test for CMV infection (IgG and IgM) . If primary infection was diagnosed a complete follow up program was performed (monthly high resolution ultrasound, elective amniocentesis and fetal magnetic resonance). If fetal infection was confirmed treatment with CMV hyperimmune globulin was also offered. All newborns were studied at birth and, if congenital CMV infection was confirmed, they entered a complete follow up programme and, when indicated, were treated with valganciclovir.

Results:

9491 woman were screened. Seroconversion rate in our population was 0,46%. Among them 81,8% had a previous child younger than three years old. Amniocentesis was performed to 21 woman, and in six cases fetal infection was confirmed. Three women had an elective abortion. Five were treated with immunoglobulin. Four women were lost to follow up.

38 newborns were studied at birth. 14 of them were diagnosed of congenital CMV infection. One of them showed unilateral deafness and severe impairment on cranial ultrasound and MRI findings. Another one presented with unilateral neurosensorial hearing loss. Both patients received valganciclovir for six months. Among the others, nine presented minor transfontanellar ultrasound findings and all but two were treated with oral valgancyclovir. All those newborns are still asymptomatic at follow up (data summary in table 1)

Conclusions:

CMV is an important issue in perinatal pathology, and we still have to find the real consequences of congenital infection.

Screening during pregnancy might give us important preventive, prognostic and therapeutic information.

Table 1

|_Patient _|_SC trim_|_A/R_|_IUIG/dosis_|_Ab IU US/MRI _|__Neonatal BUS__|__ AER __|_ Neonatal T_|_Outcome_|

|___1____|__ 2d ___|_ No_|___ No ____|____ No _____|_LS vasculopathy_|_Normal_|__6 weeks___|_ normal __|

|___2____|__ 2d ___|_ No_|___ No ____|____ No _____|___ Normal _____|Abnormal|__ 6 months _|_ normal __|

|___3____|__ 2d ___|Yes: +|__Yes/1____|____ No _____|_LS vasculopathy_|_Normal_|__6 weeks___|_ normal __|

|___4____|__ 3d ___|_ No_|___ No ____|____ No _____|_LS vasculopathy_|_Normal_|__6 weeks___|_ normal __|

|___5____|__ 1st ___|Yes: +|__Yes/1____|____ No _____| Germinolytic cyst |_Normal_|__6 weeks___|_ normal __|

|___6____|__ 3d ___|_ No_|___ No ____|____ No _____|_LS vasculopathy_|_Normal_|__4 weeks___|_ normal __|

|___7____|__ 2d ___|Yes: - |___ No ____|____ No _____|___ Normal _____|_Normal_|__3 weeks___|_ normal __|

|___8____|__ 2d ___|Yes: - |___ No ____|____ No _____| Germinolytic cyst |_Normal_|__3 weeks___|_ normal __|

|___9____|__ 1st ___|Yes: +|___ No ____|____ No _____| Germinolytic cyst |_Normal_|__6 weeks___|_ normal __|

|__ 10 __ _|__ 3d ___|_ No_|___ No ____|____ No _____|___ Normal _____|_Normal_|___ No _____|_ normal __|

|___11 ___|__ 3d ___|_ No_|___ No ____|____ No _____|___ Normal _____|_Normal_|___ No _____|_ normal __|

|___12 ___|__ 3d ___|_ No_|___ No ____|____ No _____|_LS vasculopathy_|_Normal_|__6 weeks___|_ normal __|

|___13 ___|__ 1st ___|_ No_|___ No ____|____ No _____|_____* _____|Abnormal |__ 6 months _|___ **____|

|___14 ___|__ 2d ___|Yes: +|__Yes/2____|____ No _____|_____ *** _____|_Normal_|__ 6 months _|_ normal __|

SC: seroconversion

A/R; amniocentesis/result

IUIG: intrauterus inmunoglobuline

Ab IU US/MRI: Abnormal intrauterus ultrasound/magnetic resonance imaging

Neonatal BUS: neonatal brain ultrasound

AER: acustic evoked response

Neonatal T: neonatal treatment

LS vasculopathy: lenticulostriate vasculopathy

* Calcifications, LS vasculopathy, White matter hyperechogenicity

** Neurological developmental delay, Cerebral palsy; Unilateral deafness

*** Caudao-thalamic groove hyperechogenicity

CMV in Europe

ANTENAL ULTRASOUND AND MRI FINDINGS OF CONGENITAL CYTOMEGALOVIRUS INFECTION (ID 071)

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Abstract

INTRODUCTION: Primary cytomegalovirus (CMV) infection occurs in 1–2% of the pregnancies, resulting in symptomatic infection at birth in 10–15% of the cases. The sensitivity of fetal ultrasound abnormalities for symptomatic congenital CMV infection is 15%, while the specificity is 94%. The aim of our study was to ascertain the prenatal ultrasound and MRI findings secondary to congenital CMV infection.

METHODS: This is a retrospective analysis of prospectively collected data on all cases with confirmed prenatal or postnatal diagnosis of congenital CMV referred to our tertiary fetal medicine unit at St George's hospital, London over a 15-year period.

RESULTS: The analysis included 33 pregnancies with congenital CMV infection. Ultrasound abnormalities were reported in 24 cases (72.7%). Nineteen out of 24 fetuses (79.2%) presented extra-cranial abnormalities. The commonest abnormalities were: echogenic bowel (47.4%), IUGR (42.1%), cardiomegaly (26.3%) and pericardial effusion (21.0%). Sixteen fetuses (66.7%) presented with ultrasound brain abnormalities, in particular 10 (62.5%) cases had ventriculomegaly, 8 (50.0%) had microcephaly, and 4 (25.0%) had hypoplastic cerebellum. MRI confirmed the ultrasound brain findings in all cases and added additional information in 10 cases (83.3%). The most frequent lesions detected with MRI were ventriculomegaly (83.3%), white matters abnormalities (58.3%), gyration abnormalities (33.3%) and microcephaly (33.3%).

CONCLUSION: The most frequent ultrasound features detected in utero were echogenic bowel, IUGR, cardiomegaly, pericardial effusion, ventriculomegaly, microcephly and hypolastic cerebellum. About 40% of white matters abnormalities detected with ultrasound and fetal MRI were located in the temporal and occipital lobes, suggesting that these could be specific targets of congenital CMV infections.

Large prospective studies are needed in order to ascertain the correlation between each of these abnormalities and the long term neurodevelopmental outcome.

CMV in Europe

10 YEARS CONGENITAL SURVEY IN FRANCE AND DATABASE EVOLUTION(ID 083)

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Abstract

In the absence of CMV screening, collecting data about the real burden of congenital cytomegalovirus infection in France remains difficult. As a reference center we put in place a systematic declaration of congenital CMV cases since 2006. We collected 797 cases between 2006 and 2015, from 28 prenatal diagnosis centers of 23 metropolitan and several overseas regions covering quite all the French territory. Since 2014, the involvement of a research assistant to better document and collect cases increased the data collected as presented below. We also put in place a systematic inquiry, derived from the previous ECDC declaration sheet, to collect data from pregnancy up to 10 years post natal follow-up, and a biological sample collection. This data base is now functional in an on-line easier-to-fill version to collect data from all French territories. Data collected include both serology, echography, RMN, clinical and follow-up data. The data base obtained the agreement of the French ethical comittees, CCTIRS and CNIL in 2015.

Case definition: congenital infection diagnosed by either amniotic fluid PCR or culture during pregnancy or viruria at birth.

Classification of cases: cases were classified on the basis of symptoms detected either by sonographic examination or at birth. Severe : neurologic abnormalities, Mild: extra-neurologic symptoms, Asymptomatic: absence of sonographic or clinical symptoms, Not documented.

Results : We collected 356 cases from 2006 to 2010, and 441 cases from 2011 to 2015, with a decrease in “not documented” cases (42% to 27%). Interestingly, the severe (22% to 25%) and moderate cases (18% and 18%) remained stable, indicating that the symptomatic cases were quite constantly declared and an increase in the asymptomatic cases (18% to 30%) showing improvement in the number of recognized or declared infections. In 2015, 24% were asymptomatic, 59% symptomatic, including 78% severe cases, i.e. with neurological abnormalities and 17% not documented. Amongst the 60

symptomatic newborn from 2015 there were 1 death (2%), 1 death in utero (2%), 14 medical abortions (23%), 23 hearing loss (49%), 1 neurological abnormality (2%), 7 mental disabilities (15%). (Detailed and updated results will be presented). This proportion is in agreement with previous clinical description of congenital CMV. However, the asymptomatic cases are still under declared and possibly under recognised, and thus cannot receive the recommended prolonged follow-up.

In 2016, cases report can be easily filled directly in the On Line data base, an evolving data base on a Voozanoo™ platform (Epiconcept). Maternal, fetal and newborn data are collected, with demographical, biological and clinical documentations. Under the coordination and monitoring of the National Reference Center, data will be entered by both virologists, gynecologists and paediatricians (possibly neurologists, ophthalmologists, ENT specialists) with personal and secure access while avoiding duplicates. Moreover, it can be connected to other data bases, and can be used as a a tool for further clinical studies or dedicated retrospective data research, provided the agreement of all investigators.

We hope this will facilitate and enlarged the case declaration, leading to a better knowledge of congenital CMV burden and consequences in France and overseas.

CMV in Europe

CYTOMEGALOVIRUS SEROPREVALENCE AND SEROCONVERSION RATES IN A POPULATION-BASED PREGNANCY COHORT STUDY IN NORWAY (ID 096)

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Abstract

Brief introduction: Preventive measures to reduce the incidence of congenital CMV-infections have to be based on assessment of risk factors for maternal primary CMV-infection. We have thus carried out a study to estimate the seroprevalence and seroconversion rates of CMV according to age and socioeconomic factors in a prospective population-based pregnancy cohort study in Norway. Methods: The Norwegian Mother and Child Cohort Study (MoBa) includes 114 000 children and 95 000 mothers recruited from all over Norway from 1999-2008. Serum samples and questionnaires from 1349 randomly selected pregnant women in the MoBa cohort were included. Sera collected at birth (sample K2) were analysed for CMV IgG and IgM using an enzyme linked immunosorbent assay (Virion/Serion, Würzburg). All samples which did not have a serological profile consistent with CMV IgG and IgM negative status were analysed for CMV IgG and IgM around week 17-18 (sample K1). CMV-IgG avidity analyses (Euroimmun, Lubeck, Germany) was performed if both IgG and IgM were positive in K1. Umbilical cord blood from children with IgG positive mothers in K2 where analysed with an in-house real-time CMV PCR. Results: The K2 sample was anti-CMV IgG positive in 56% and negative in 44% of the women. 28 of them (4.5%) seroconverted, which gives an annual seroconversion rate of 10.2 %. Only 2 of 28 women had positive IgM in K2, and they both gave birth to children that were CMV PCR positive. In addition, a woman with a non-primary infection gave birth to a CMV PCR positive child. In total 3 (0.22%) children had a positive CMV PCR in umbilical cord blood. Analysis of CMV-IgG avidity index in 28 (2.1 %) women showed that 4 women had low and 2 had borderline avidity in K1 and a significant increase in K2 indicating a primary infection. Mean age at delivery was 30.4 years and 40.2% was 29 year or younger. Mean gestational age at delivery was 39.7 weeks. 47.1% was nulliparous. 30.5 % of the mothers had≤12 years, and 69.5 % had ≥13years of formal education. 75% had children in household <6 years and 65.3% had at least one child in day-care centre. The seroprevalence of CMV-IgG antibodies was significantly higher in

women with low education and previous pregnancies. Seroconversion was not linked to any of the socioeconomic factors studied. Conclusions: Factors like education and parity influence the CMV-IgG seroprevalence in pregnant Norwegian women. Age seems not to influence the seropositivity in pregnant Norwegian women indicating that the major transmission of CMV occurs at a younger age.

CMV in Europe

INVESTIGATION THE GENOTYPES OF STRAINS IN UL55 AND UL75 REGIONS IN VARIOUS CLINICAL CYTOMEGALOVIRUS (CMV) SPECIMENS (ID 109)

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Abstract

Cytomegalovirus (CMV) in immune compremized patients causes symptomatic and asymptomatic infection which is a virus belonging to the herpesviridae family. These patients especially include transplant patients, hemotology patients and AIDS. It is known that CMV genome have a large number of variations and consequently different genotypes emerge. Common genotypes involve genetic polymorphisms gB (UL 55) and gH (UL 75) and are in the envelope glycoproteins. In our study, 50 CMV (+) patients was included in phylogenetic analysis with gB and gH gene region. CMV DNA isolation and amplification are performed with the use of specific primers. After the PCR process, DNA sequence analysis was performed and the results are evaluated with 6.0 MEGA program and phylogenetic tree was created.

According to the results of phylogenetic analysis; from the total of 50 patients tested only 48 patients yielded results for the gB gene region. Patients' gB (UL55) genotype distribution by region was as follows: gB1 genotype 25 (50%), gB2 genotype 7 (14%) and 16 gB3 genotype (32%). UL 75 (gH) gene amplification has been done successfully in all of the 50 patients; according to the results of phylogenetic analysis, there were 6 (12%) gH1 patients and 44 (88%) patients of the gH2 genotype.

In our country, the CMV genotyping studies involving gB gene usually found that gB1 genotype is the dominant gene. The dominant gB type found in our study is consistent with other studies which is found to be gB1. In the region of gH gene, the dominant was the gene type gH2. This study demonstrated the prevalence and role of HCMV genotypes in infection and disease in various clinical patients in Turkey.

Table / Image

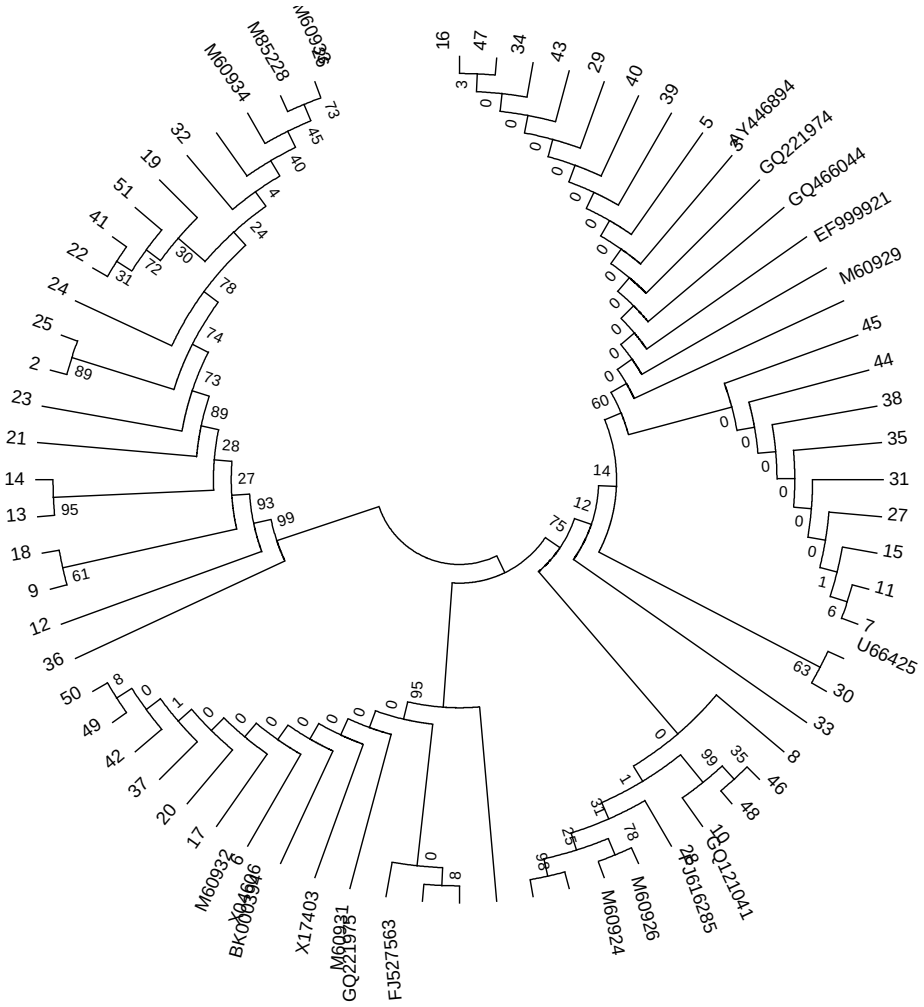

Image: Pylogenetic analysis of patients in gB gene

		Genotype (n/%)			
Region of Gene	**Number of Patiens (n)**	**1**	**2**	**3**	**4**
UL55 (gB)	50	25 (50%)	7 (14%)	16 (32%)	-
UL75 (gH)	50	6 (12%)	44 (88%)		

Table: Genotypes of patients

CMV in Europe

IN VITRO EVALUATION OF FOUR BODIES ANTIBODIES INFECTION AND PROPAGATION OF CVM ISOLATES (ID 112)

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Abstract

The potential of anti-CMV antibodies directed against the human CMV gB to reduce viremia in the clinical setting has been suggested by the gB vaccine studies. However, the neutralizing potential may differ from one antibody to another and other antibodies directed against glycoproteins pentamers have been associated to reduced viraemia. The place of the anti gB thus deserves further analysis. To better understand their possible effect, we tested several antibodies against CMV infection and replication, using both free virion stocks and a cellular inoculum to test neutralization of infection and inhibition of cell-to-cell virus propagation. TRL345 is a native monoclonal anti-gB antibody developed by Trellis BioScience, LLC. Its potential to neutralize infection and spread of virus in MRC-5 human fibroblasts was assayed in vitro in two laboratories. AD169, Toledo and Merlin strains, four clinical isolates from transplant recipients and 10 congenital CMV isolates, with different gB genotypes, were used to perform neutralization assays in fibroblasts. A commercial polyclonal IgG antibody, CytoGam, obtained from serum with high neutralizing titers to CMV was used as a positive control. An irrelevant IgG (Ir) was used as negative control and a chimeric monoclonal IgA anti gB (Towne) from "B Cell design" laboratory was also assayed as a comparator.

Results: 1) toxicity : Assay performed on cells without virions or infected cells and with up to 100µg/mL of antibody (TRL345, CytoGam, IgA and irrelevant IgG) during 14 days showed no toxicity. 2) neutralization of infection by cell-free virions : Using cell-free virion stocks from

congenital CMV isolates, unit forming colonies were counted at J12 post inoculation. MAb345 at 2.8 µg/ml final is efficient to completely neutralize the viral growth of 9 clinical strains as well as that of the laboratory strain AD169, all used at a MOI less than 0.01. For one clinical strain (MOI 0,1, gB3) viral growth was only completely neutralized at higher dilutions. 3) neutralisation of infection with cell-associated virions : Using a standardized titered cellular inoculum, at a 0,02 MOI, IC50 by titer reduction assays 5 days and 14 days post infection with infected cells by counting plaque forming units were 12ug/mL, >200ug/mL, >200ug/mL and 5ug/mL for TLR345, Cytogam, Ir, and the IgA, respectively, for Toledo strain, and 6ug/mL, >200ug/mL, >200ug/mL and 4ug/mL for Merlin strain. At MOI0,002, IC50 decreased by one log, but IC90 was not reached though it was reached with ganciclovir (GCV). Results from transplant recipients isolates at MOI 0,01 were similar to Merlin or Toledo for the gB1 and gB2 isolates. However, the gB3a isolate showed a 5fold increase of IC50 (but a low IC50 for GCV).

Interestingly, at 14 days after infection a clear reduction of foci size was observed with TLR345 only, even if the number of plaques was decreasing slowly, suggesting inhibition of cell-to-cell propagation by this antibody.

In conclusion: TLR345 anti gB Mab and the anti gb chimeric monoclonal Iga demonstrated a good capacity to inhibit infection by cell-free and cell-associated virus. Moreover TLR 345 inhibits the propagation of cell-associated virus. However TLR345 and Cytogam IC50 should be compared regarding the proportion of specific anti gB antibodies in both preparations. And the neutralization capacity regarding the gB genotype or subtype has to be explored on more isolates.

CMV in the (non)pregnant woman

INTERFERON SIGNALING GENES AND CMV INTRAUTERINE TRANSMISSION
(ID 068)

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Abstract

Introduction:

CMV intra-uterine transmission occurs in only 40% of pregnancies with primary maternal infection, and the mechanism(s) involved in transmission are not clearly understood. Currently the only reliable method to diagnose fetal infection is invasive amniocentesis. Therefore, there is an urgent need for an early and non-invasive assay which can predict transmission of the virus.

Methods:

A mathematical model for stimulation of interferon (IFN) signaling genes in response to CMV infection based on data from five published array-datasets, led us to suggest seven biomarker genes for predicting intrauterine-transmission of CMV. Validation of our prediction-model was performed by qRT- PCR on PBMC's RNA samples from 26 pregnant women with primary CMV infection. Transmission was determined by qRT- PCR of CMV in amniotic fluid or newborn urine.

Results:

Combined analysis of data of IFN signaling genes before and after CMV stimulation from five GEO microarray datasets revealed two distinct sub-populations; those with high basal expression and low rise upon stimulation and those with low basal expression and high rise upon stimulation. This phenomenon may reflect a more efficient immune response with a robust increase during acute infection and a rapid decline to low basal state thereafter. Seven of these genes were selected for analysis of the expression of the IFN signaling genes on 26 pregnant women with primary CMV infection who were viremic (minimum of 200 CMV copies/ml blood). We found that women with

high basal expression of the tested genes were significantly more likely to transmit the virus to the fetus.

In our cohort, the IFN signaling genes levels predicted transmission with a sensitivity of 92.3% (95% CI: 72, 92), specificity of 100% (95%CI: 80, 100), PPV of 100% (95% CI: 78, 100) and NPV 92.9% (95% CI: 74, 93).

Conclusions:

Low basal expression of the assayed IFN signaling genes is associated with low likelihood of maternal fetal infection. Further research with larger cohort might facilitate the clinical utilization of our data.

CMV in the (non)pregnant woman

COMBINATION OF LINE IMMUNOASSAYS MIKROGEN RECOMLINE CMV IGG AND RECOMLINE CMV IGG AVIDITY HELPS TO DATE THE ONSET OF CMV PRIMARY INFECTION (ID 087)

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Abstract

Objectives: 1. To assess the ability of the line immunoassays Mikrogen RecomLine CMV IgG and RecomLine CMV IgG Avidity to date the onset of CMV primary infection comparing to VIDAS CMV IgG Avidity. 2. To evaluate the added value of the combination of RecomLine CMV IgG and RecomLine CMV IgG avidity compared to VIDAS CMV IgG Avidity

Study design: 1. For the first objective, a panel of 46 sequential sera collected from 29 women with precisely determined onset of CMV primary infection was tested with VIDAS CMV IgG Avidity, Mikrogen RecomLine CMV IgG and RecomLine CMV IgG. 28 serums were drawn within 12 weeks of infection and 18 were obtained more than 12 weeks after infection. 2. A second panel of 51 sera collected from pregnant women presenting with positive CMV IgM and a borderline avidity with VIDAS was analysed.

Results: 1. Among the 28 sera ≤ 12 weeks of infection, VIDAS CMV IgG Avidity showed a reliable low avidity result in 19 cases, a borderline not contributory result in 8 cases and a false positive high avidity in 1 case. The combination of RecomLine CMV IgG and RecomLine CMV IgG avidity showed 24 reliable results with the following interpretation: n=6 <6-8 weeks, n=1 between 6-8 and 14 weeks, n=15 12 weeks of infection, VIDAS gave 13 reliable high avidity results and 5 borderline non-contributory results. The combination of RecomLine CMV IgG and RecomLine CMV IgG avidity gave 14 reliable results (n=13 >12weeks, n=1 >24 weeks), 1 infection <14 weeks and 3 non-contributory positive IgG.

2. Among the 51 sera with a borderline avidity with VIDAS, the combination of line immunoassays yielded following results: 4 infections 6-8 weeks without additional statement, 6 infections between 6-8 and 14 weeks, 6 infections >12 weeks, 1 infection >24 weeks, 20 infections <14 weeks and 10 cases of non-contributory positive IgG.

Conclusion: The combination of Mikrogen RecomLine CMV IgG and RecomLine CMV IgG avidity showed a good reliability to date CMV infection and less non-contributory results than VIDAS CMV

IgG Avidity. Among a panel of non-contributory results with VIDAS CMV IgG Avidity, this combination helped to date the onset of infection in 80% of cases.

CMV in the (non)pregnant woman

EVALUATION OF CMV PRENATAL DIAGNOSIS IN PREGNANT WOMEN UNAWARE OF THEIR SEROSTATUS ADMITTED TO POLICLINICO HOSPITAL-BARI -ITALY. (ID 089)

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Abstract

Introduction. To the aim to bring down the CMV congenital infection and avoid, whenever possible, invasive prenatal diagnosis (PD), the evaluation of CMV serostatus before pregnancy is strongly recommended. However, only a small percentage of women becomes pregnant being aware of their CMV immunological status.

Study design. Over the period 2010-2014, 217 pregnant women suspected for primary CMV infection were admitted at the Obstetric Ginecology Unit, Policlinico Hospital-Bari.

On the basis of clinical and laboratory records, 71/217 patients underwent to PD in the 21st week of pregnancy by RT-PCR DNA CMV on amniotic fluid (AF) at the Microbiology and Virology Unit. According to the period of seroconversion the patients were classified in the following groups: a. first trimester (n=51); b. second trimester (n=7); c. third trimester of pregnancy (n=2); d. periconceptional age (n=11).

Furthermore, anti-CMV specific hyperimmunoglobulin therapy (HIG) was administrated to 17/71 (23.9 %) women.

Results. Group a. 47/51 (92.1%) women were AF negative. 11/47 were treated with HIG. The outcome was 46 healthy children (CMV viruria negative); 1/47 patient who received HIG underwent voluntary interruption of pregnancy (VIP) in the 22nd week because of anomaly scan. Of the 4 AF positive women, 2/4 chose VIP (AF viral load >106 copies/ml) and 2/4 carried the pregnancy to term giving birth to 1 asymptomatic CMV congenital infected newborn (mother treated with HIG) and 1 CMV congenital infected newborn with unilateral deafness, respectively.

Group b. 4/7 women were AF negative. At birth 2/4 newborns were healthy and 2/4 (one from HIG treated mother) were asymptomatic CMV congenital infected. Of 3 AF positive women, one HIG treated woman interrupted pregnancy (AF viral load >106 copies/ml); 2 gave birth CMV congenital infected newborns, 1 asymptomatic and 1 (from HIG treated mother) with hearing loss, respectively.

Group c. 2/2 women with PD carried out beyond the 22nd week of pregnancy resulted AF positive in one case and AF negative in the other. Both newborns were asymptomatic CMV congenital infected.

Group d. 11/11 women were AF negative. 1/11 stopped pregnancy because of intrauterine premature death and 2/11 were treated with HIG. In all the cases newborns were healthy.

Considerations and conclusions. All the women who were offered PD had begun the pregnancy unaware of their CMV serological status; the higher seroconversion rate was found in the first trimester of pregnancy (72.8%, group a); a major discrepancy between the AF positive results and neonatal outcomes was observed in group b. However, if in one case the viral transmission was predicted on the basis of the seroconversion time when compared to scheduled PD, in the other case it was unexpected. In this regard, it might be hypothesized that the HIG treatment delayed viral transmission.

If we consider that many variables exist that do not allow to predict the HIG therapy effects, suitable protocols with very strict inclusion criteria on a larger population are needed.

Promoting CMV awareness among the childbearing age women might represent an useful tools to both avoid unnecessary PD and reduce the risk of CMV congenital disease.

CMV in the (non) pregnant woman

IGG AVIDITY DETERMINATION TO EXCLUDE A PRIMARY HUMAN CYTOMEGALOVIRUS (HCMV) INFECTION IN PREGNANT WOMEN TESTED IN THE FIRST TRIMESTER OF GESTATION: A NEW APPROACH TO PRENATAL HCMV ANTIBODY SCREENING? (ID 090)

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Abstract

Background: Prenatal screening for human cytomegalovirus (HCMV) is not recommended in Italy. Nevertheless, about 40% of pregnant women are routinely tested for HCMV IgG and IgM antibody to assess immune status and detect subclinical infections. Since IgM detection alone is not a reliable marker of primary HCMV infection, interpretation of IgM positive results is essential. Presently, the most widely used approach to interpretation of IgM-positive results relies on IgG avidity determination. Objective: To investigate the performance of a commercially available HCMV IgG avidity assay (LIAISON® CMV IgG Avidity II, DiaSorin. Saluggia, Italy) to exclude a primary HCMV infection in pregnant women and its potential use as first line HCMV antibody testing. Methods: Overall, 506 sequential serum samples from 251 pregnant women referred within 12 weeks of gestation to our Institute for confirmation/interpretation of a positive HCMV IgM result obtained elsewhere were included in the study. A primary HCMV infection was excluded or diagnosed (and presumed onset defined) on the basis of a previously reported algorithm (Revello MG et al., J Clin Virol. 2011 50:303-7). Serum samples were also tested in parallel with the LIAISON® CMV IgG Avidity II assay. Performance of the LIAISON® CMV IgG Avidity II was assessed retrospectively on the first available serum samples (n=251). Results: A primary HCMV infection in pregnancy was excluded in 174 (69.3%) women. Of these, 5 women were HCMV IgG-negative and 169 were HCMV IgG-positive without markers of primary infection. A primary HCMV infection was diagnosed in the remaining 77 women (18 preconception and 59 in the first trimester of gestation). LIAISON® CMV IgG Avidity II assay results are summarized in the Table.

Conclusions: In this highly selected and well characterized population, LIAISON® CMV IgG Avidity II assay showed excellent specificity (99.4%) and sensitivity (98.7%) in excluding/diagnosing a primary infection in pregnancy in women tested at < 12 weeks' gestation. Had the avidity assay been used as first line test, i) immune status determination would have been

achieved, ii) further investigations for the interpretation of IgM positive results would have been safely avoided in more than 70% cases, and iii) almost the totality of the women with subclinical recent primary infection would have been correctly identified. In view of the good discriminating capacity observed, ad hoc studies to investigate performance of the LIAISON® CMV IgG Avidity II assay as first line assay for prenatal HCMV screening within the first trimester of pregnancy are warranted.

Table / Image

Type of maternal HCMV infection (presumed onset)	No. of women with IgG avidity results				
	n	High	Intermediate	Low	Not determinable
HCMV seronegative	5				5
Primary infection excluded	169	168	1	0	
Primary infection (pre-conception)	18	10	7	1	
Primary infection (1st trimester)	59	1*	22	36	

*High avidity values were also obtained with the home-brew avidity test (reference test) as well as another commercially available kit

CMV in the (non)pregnant woman

DETERMINATION OF HUMAN CYTOMEGALOVIRUS IGG ANTIBODIES ON SALIVA AND FINGER PROCK BLOOD SAMPLES (ID 094)

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Abstract

Introduction: Human cytomegalovirus (HCMV) infection is common and IgG seroprevalence in developed countries is about 50-60%. HCMV-seronegative women are at risk of infection, especially if they have contacts with children aged <3 years. Recently, a controlled study showed that an intervention based on the identification and hygiene counseling of HCMV-seronegative pregnant women significantly prevented maternal infection (Revello et al., EBioMedicine 2015, 2:1205-10).

Objective: To perform a preliminary evaluation of HCMV IgG determination using saliva and finger prick blood samples as an alternative to serum samples obtained by venipuncture.

Methods and Patients: FLOQSwabsTM (Copan Italia, Brescia Italy) were used to collect saliva and blood samples. Saliva samples were collected by putting the swab on the tongue for 5 seconds, and then by twisting the swab 10 times in each gingival sulcus. For finger prick blood samples a disposable finger prick device was used to prick the finger skin and one drop of blood was collected on a single swab. Saliva and blood swabs were air dried for at least 12 hours and then stored in conical microtubes at room temperature. For IgG antibody determination, 200-500 ul PBS solution was added; tubes were vortexed for 1 minute and incubated at room temperature for 15 minutes. Following centrifugation, swabs were removed and eluates used for serologic testing. Ten healthy donors known to be HCMV-seronegative and 30 patients were studied. HCMV IgG antibody determination was performed on serum samples (reference test) and on eluates of saliva and blood swabs by using an in-house developed ELISA.

Results: Cut-off values for IgG determination on saliva and blood samples were established on the basis of results obtained with 10 HCMV-seronegative (reference test) donors. All 30 patients were correctly identified as HCMV IgG-positive (n=22) or IgG-negative (n=8) on both saliva and whole blood samples. Thus, concordance with results obtained on serum samples was 100%.

Conclusions: These preliminary results indicate that both saliva and finger prick blood samples collected with FLOQSwabTM appear suitable for determination of HCMV-specific IgG antibodies. However, collection of saliva sample is less invasive and, thus, more acceptable. Moreover, the same sample can be used for detection of HCMV DNA shedding by real time PCR.

CMV in the (non)pregnant woman

CLINICAL EVALUATION OF THE ROCHE SEROLOGICAL AND MOLECULAR CMV SPECIFIC TESTS IN THE DIAGNOSIS AND PROGNOSIS OF CONGENITAL CMV INFECTION. (ID 114)

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Abstract

Congenital CMV infection is the most prevalent infection-related cause of congenital neurological handicap and sensorineural hearing loss.

CMV infection can occur in pregnant women by non primary infection, namely reactivation of latent virus or reinfection with a different strain, or by primary infection. Mother-child transmission is mainly the result of primary maternal CMV infection which carries a risk of transmission about 30-40% and cases of CMV transmission due to nonprimary infection have been reported in 1-2.2% of cases, i.e. at a much lower rate than those resulting from primary infection.

Prevention is almost always not implemented and considered difficult because the virus is ubiquitous and infection is common. Therefore today, laboratory techniques represent a decisive approach for management of CMV infection in pregnancy.

Given the implications of CMV diagnosis, new assays must be thoroughly characterised with well-classified specimens in order to ascertain their sensitivity and specificity and to ensure that they perform at least as well as or better than established routine tests on unselected samples.

The primary aim of this retrospective study is to evaluate the utility of the Roche serological and molecular CMV specific tests in the diagnosis and prognosis of congenital CMV infection and in supporting their appropriate clinical management.

In particular, we evaluated i) the Elecsys CMV IgM, IgG and Avidity test in the diagnosis of primary CMV infection during the first part of pregnancy (within 14-16 weeks gestation); ii) the COBAS

AmpliPrep/COBAS TaqMan CMV test in the diagnosis and prognosis of fetal CMV infection testing amniotic fluid samples collected at 20-21 weeks of gestation from pregnant women with ascertained primary CMV infection arising before the14-16 weeks of gestation; iii) the COBAS AmpliPrep/COBAS TaqMan CMV test in the diagnosis of neonatal CMV infection testing urine samples collected within the first 2 weeks of life.

We selected a group of 50 serum samples with CMV IgG and IgM positive results and low/moderate IgG avidity for CMV obtained from 50 pregnant women with ascertained primary CMV infection arising before the 14-16 weeks of gestation.

From this same group of pregnant women, we evaluated 50 amniotic fluid samples (5 CMV-negative and 45 CMV-positive) collected at the time of amniocentesis (20-21 weeks of gestation), all pregnant women underwent ultrasound examinations that included a survey of all fetal organs.

Finally, we tested 50 urine samples collected within the first 2 weeks of life from 50 newborns: 5 urine samples collected from 5 CMV-uninfected newborns and 45 urine samples collected from 45 CMV infected newborns with or without symptoms.

Correlation among different tests and the positive and negative predictive values (PPV and NPV) of fetal and newborn CMV disease will be estimated using statistical methods.

The interim analysis of the results obtained with the Roche platform that includes the Elecsys CMV IgM, IgG and Avidity test and the COBAS AmpliPrep/COBAS TaqMan CMV test compare well with routinely used tests, and, as such, are suitable for clinical use.

CMV in the fetus

PRNATAL AND POSTNATAL NEUROIMAGING FOR THE PREDICTION OF OUTCOME IN FETUSES WITH CONGENITAL CYTOMEGALOVIRUS INFECTION. (ID 059)

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Abstract

Background: To examine the clinical outcome of children with proven and well-dated cytomegalovirus (CMV) infection during fetal life and to investigate the possible relationship between prenatal and postnatal neuroimaging findings and postnatal development.

Methods: The prenatal and postnatal records of 23 cases with congenital CMV infection were retrospectively reviewed. Fetal infection was ascertained by amniotic fluid analysis in all but one case, which was diagnosed soon after birth. Obstetric ultrasound examinations were performed every 4 weeks until the term of pregnancies in all women. All fetuses underwent brain magnetic resonance imaging (MRI) and in 9 of these cases MRI was carried out twice, at 21 and 32 weeks' gestation. All neonates underwent clinical examination, including hearing and eye fundus examination, brain ultrasound and MRI scans, and they were followed-up by specialists in child development.

Results: On the total of 23 fetuses, primary CMV infection occured during the periconceptional period in 3 cases, during the first trimester in 13 and in the second trimester of pregnancy in 6 fetuses. In 1 case, re-infection occurred during the first trimester.

In 5 cases the parents decided for termination of pregnancy and there was miscarriage following the invasive procedure in 2 cases, leaving a population of 16 fetuses with postnatal clinical follow-up.

Obstetric ultrasound showed a normal appearance of the fetal brain in all 16 cases, with mild unilateral ventriculomegaly (atrial width of 13 mm) in 1 fetus. Fetal brain MRI confirmed isolated ventriculomegaly and showed signs of mild cerebellar bleeding in another fetus. Subtle findings, such as cerebellar biometry on the lower limits of the normal range, subependymal cysts, enlargement of the subarachnoid space and white matter signal hyperintensity, were reported in additional 5 cases. Postnatal brain MRI confirmed the prenatal findings and provided additional information in 7 cases, including white matter signal hyperintensity, signs of mild cerebellar bleeding and periventricular cysts. Overall, postnatal neuroimaging showed abnormalities of some kind in 14 (87.5%) of 16 children. Clinical follow-up at a mean age of 2 years (range 1-5 years) showed a normal neurodevelopment in all cases. At the time of writing, 1 child has unilateral deafness and another has mild unilateral hearing impairment. In the first case, fetal MRI at 23 weeks was normal and postnatal MRI showed white matter hyperintensity. In the second case, fetal MRI showed cerebellar biometry on the lower limits of the normal range and cerebellar hypoplasia was reported on postnatal MRI.

Conclusions: In this small cohort of children with congenital CMV infection ascertained during fetal life, the incidence of minor abnormalities on postnatal brain MRI was very high and these were not clinically useful to predict neurodevelopment or hearing impairment. Therefore, fetal MRI, which detected 50% of such findings in our series, seems to be of limited value in prenatal counselling and it may increase parental anxiety in the presence of abnormalities of uncertain clinical significance.

CMV in the fetus

ULTRASOUND BRAIN ABNORMALITIES IN FETUSES WITH CONGENITAL CYTOMEGALOVIRUS INFECTION; SISTEMATIC REVIEW AND META-ANALYSIS. (ID 072)

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Abstract

Introduction: The incidence of fetal abnormalities associated with congenital CMV infection is likely to be overestimated due to bias in reporting cases where fetal abnormalities detected on ultrasound triggered testing for CMV. The main aim of this study was to quantify fetal brain ultrasound abnormalities secondary to congenital CMV infection.

Methods: Medline, Embase and the Cochrane library were searched electronically utilizing combinations of the relevant medical subject heading for ""Cytomegalovirus infection" and "ultrasound". Studies reporting data on the prenatal brain ultrasound findings in fetuses with confirmed congenital CMV infection were included. The outcomes assessed included brain abnormalities associated with congenital CMV infection, such as intracranial calcifications, ventriculomegaly, microcephaly, intracranial cyst, intraventricular septa, cerebellar dysplasia-hypoplasia, brain haemorrhage, lenticulostriate vasculopathy, sulcation or gyral abnormalities and white matters abnormalities. Two authors reviewed all abstracts independently. Quality assessment of the included studies was performed using the Newcastle-Ottawa Scale (NOS) for cohort studies. Meta-analyses of proportions were used to combine data. Between-study heterogeneity was explored using the I2 statistic.

Results: A total of 982 articles were identified, 48 were assessed with respect to their eligibility for inclusion and total of 7 studies were included in the systematic review. The most common brain ultrasound abnormalities detected during the second trimester of pregnancy were: white matter abnormalities (17.6%), ventriculomegaly (9.3%), microcephaly (2.7%) and brain cysts (2.7%). Less common were abnormal cerebellar findings and cortical migration abnormalities (0.4% and 1.4% respectively).

The most common brain ultrasound abnormalities detected during the third trimester of pregnancy were: brain calcification (31.5%), ventriculomegaly (26.6%) and white matter abnormalities (29.3%).

Less common were microcephaly (8.1%), brain cyst (4.9%), intraventricular synechiae (8.7%) and cortical migration abnormalities (8.7%).

Conclusions: The most frequent ultrasound brain lesions associated with congenital CMV, both in second and third trimester of pregnancy, are white matter abnormalities. The higher prevalence during the third trimester highlights the importance of serial targeted brain examination. Large prospective studies are needed in order to ascertain the correlation between each of these abnormalities and the long term neurodevelopmental outcome.

CMV in the fetus

ULTRASOUND EXTRA-CRANIAL FETAL ABNORMALITIES IN CONGENITAL CYTOMEGALOVIRUS INFECTION : SISTEMATIC REVIEW AND META-ANALYSIS. (ID 073)

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Abstract

Introduction: Several studies reported that the presence of prenatal fetal abnormalities is an independent predictor for a poor outcome. However, the incidence of fetal extra-brain ultrasound abnormalities secondary to congenital CMV infection is yet to be established. The main aim of this study was to quantify fetal extra-cranial ultrasound abnormalities secondary associated with CMV infection.

Methods: Medline, Embase and the Cochrane library were searched electronically utilizing combinations of the relevant medical subject heading for "“Cytomegalovirus infection” and “ultrasound”. Studies reporting data on the prenatal brain ultrasound findings in fetuses with confirmed congenital CMV infection were included. The outcomes assessed included brain abnormalities associated with congenital CMV infection, such as intra-uterine growth restriction (IUGR), echogenic bowel, pericardial effusion, hydrops, ascites, cardiomegaly, hepatomegaly, placentomegaly, placental calcifications, hepatic calcifications, polyhydramnios, oligo-anyhydramnios. Two authors reviewed all abstracts independently. Quality assessment of the included studies was performed using the Newcastle-Ottawa Scale (NOS) for cohort studies. Meta-analyses of proportions were used to combine data. Between-study heterogeneity was explored using the I2 statistic.

Results: A total of 982 articles were identified, 48 were assessed with respect to their eligibility for inclusion and total of 6 studies were included in the systematic review. The most common extra-cranial ultrasound abnormalities detected during the second trimester of pregnancy were: echogenic bowel (7.7%) and oligohydramnios (3.9%). Less common abnormalities were ascites (2.0%), IUGR (2.5%) and hepatic calcifications (0.4%). The most common extra-cranial abnormalities detected during the third trimester of pregnancy were: IUGR (17.9%), echogenic bowel (11.5%), ascites (11.2%), and placentomegaly (11.2%). Other extra-brain abnormalities, including pericardial effusion, hepatomegaly, hepatic calcifications, cardiomegaly and oligohydramnios were demonstrated in less than 10% of these fetuses.

Conclusions: The prevalence of extra-brain abnormalities is higher in the third trimester, and the most common abnormalities were IUGR (18%) and echogenic bowel (12%). These findings highlight the importance of serial targeted ultrasound examination during the third trimester of pregnancy. Large prospective studies are needed in order to ascertain the correlation between each of these abnormalities and the long term outcome.

CMV in the fetus

Brain MRI Abnormalities in Fetuses with Congenital Cytomegalovirus Infection: Systematic Review and Meta-analysis (ID 074)

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Abstract

Introduction: It is widely accepted that fetal MRI is complementary to ultrasound brain evaluation, and is likely to increase the sensitivity and positive predictive value for the identification of fetuses that will develop symptomatic congenital CMV infection. However, the incidence of fetal brain MRI abnormalities secondary to congenital CMV infection is yet to be established. The aim of this study was to quantify the incidence of fetal brain MRI abnormalities secondary to congenital CMV infection.

Methods: Medline, Embase and the Cochrane library were searched electronically utilizing combinations of the relevant medical subject heading for ""Cytomegalovirus infection" and "MRI". Studies reporting data on the prenatal brain MRI findings in fetuses with confirmed congenital CMV infection were included. The outcomes assessed included brain abnormalities associated with congenital CMV infection, such as intracranial calcifications, ventriculomegaly, microcephaly, intracranial cyst, intraventricular septa, cerebellar dysplasia-hypoplasia, brain haemorrhage, lenticulostriate vasculopathy, sulcation or gyral abnormalities and white matters abnormalities. Two authors reviewed all abstracts independently. Quality assessment of the included studies was performed using the Newcastle-Ottawa Scale (NOS) for cohort studies. Meta-analyses of proportions were used to combine data. Between-study heterogeneity was explored using the I2 statistic.

Results: A total of 119 articles were identified, 14 were assessed with respect to their eligibility for inclusion and total of 7 studies were included in the systematic review. Among the 252 fetuses of congenital CMV infection that had an antenatal brain MRI, there were only 2 cases with brain calcifications, giving an overall prevalence of 1.2% (95% CI, 0.2-3.0). The prevalence of ventriculomegaly was 13.0% (95% CI, 5.8 – 22.6). Fifteen out of 252 fetuses were diagnosed with microcephaly (4.9%, 95% CI, 0.9-11.8). The overall incidence of brain cysts was 8.5%. Abnormal cerebellar findings, described as cerebellar hypoplasia or cerebellar dysplasia, were described in only 5 out of 252 cases of congenital CMV, (2.2%, 95% CI, 0.8-4.4). White matter abnormalities and cortical migration abnormalities had an incidence of 32.8% and 6.5%, respectively.

Conclusions: The most frequent brain lesions reported in fetuses with congenital CMV undergoing prenatal MRI evaluation are white matter abnormalities (33%), especially in the temporal lobes. The second most common brain abnormalities reported is ventriculomegaly (13%). Large prospective studies are needed in order to ascertain the correlation between each of these abnormalities and the long term neurodevelopmental outcome.

CMV in the fetus

CYTOMEGALOVIRUS CONGENITAL INFECTION: PROGNOSTIC VALUE OF MATERNAL DNAEMIA AT TIME OF AMNIOCENTESIS (ID 081)

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Abstract

INTRODUCTION:

Primary human Cytomegalovirus (HCMV) infection during pregnancy can lead to congenitally infection in approximately 30% of cases, with 10–15% of symptomatic disease birth and 10–15% of neurological sequelae in asymptomatic newborns. Antenatal care are currently unable to identify infants who will develop symptomatic disease and antenatal counselling encounters several difficulties due to the absences of antenatal prognostic markers of neonatal damage.

ABSTRACT

The aim of our study was to define if maternal DNAemia in peripheral blood of pregnant women with primary HCMV infection at the time of amniocentesis can reveal a prognostic value in term of congenital infection and neonatal symptomatic disease.

This is a prospective observational study of pregnant women referred to our Maternal-Fetal Medicine Division with suspect of HCMV infection from January 2007 to December 2014. Primary infection was diagnosed based on seroconversion for HCMV and/or HCMV IgM-positive and low/moderate HCMV IgG avidity, after determination of specific IgM and IgG by automated chemiluminescence immunoassay and confimatory immunoblot. Amniocentesis was performed after 20 weeks of gestation and at least 6 weeks after maternal infection, amniotic fluids were subjected to a direct search for HCMV in culture and for the viral genome by real-time polymerase chain reaction (PCR). Maternal whole blood samples were analyzed by real-time PCR to determine maternal DNAemia.

With respect to fetal/neonatal outcome, newborns were classified as uninfected or infected on the basis of virus isolation and real-time PCR from urine sampled within the first two weeks of life, and in symptomatic or asymptomatic disease on the basis of fetal autopsy or pediatric follow-up.

Two hundred and fourty-one pregnant women were enrolled: 32 blood samples (13.3%) were positive and 209 (86.7%) negative for the detection of HCMV-DNA. Overall rate of HCMV transmission was 23.6% (57 fetuses/newborns). Among infected case, 8 aborted fetuses and 7 newborns (26.3%) were defined symptomatic and the remaining 9 fetuses and 33 newborns (73.7%) were asymptomatic. Vertical transmission occurred in 14 (43.8%) women with positive HCMV-DNAemia and in 43 (20.6%) women with negative HCMV RT-PCR in the blood (p-value 0.05).

CONCLUSION

In our sample, PCR DNA performed at the time of amniocentesis seems to be significantly correlated with congenital infection, with a 3 fold increased risk of vertical transmission, but it did not show correlation with the presence of congenital symptomatic disease at birth or at neonatal follow up.

CMV in the fetus

CYTOMEGALOVIRUS CONGENITAL INFECTION: PROGNOSTIC VALUE OF MATERNAL DNAEMIA AT AMNIOCENTESIS (ID 091)

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2. Department of Specialised, Experimental, and Diagnostic Medicine, Division of Microbiology and Virology, St. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

Abstract

INTRODUCTION

Primary human cytomegalovirus (HCMV) infection during pregnancy can lead to congenital infection in approximately 30% of infants. Of these 10–15% have symptomatic disease birth while 10–15% of asymptomatic newborns have neurological sequelae. Current antenatal care cannot identify infants who will develop symptomatic disease and counselling is hampered by the lack of antenatal prognostic markers of neonatal damage.

ABSTRACT

The aim of our study was to ascertain if DNAemia in the peripheral blood of pregnant women with primary HCMV infection at the time of amniocentesis may have a prognostic value in terms of congenital infection and neonatal symptomatic disease.

This was a prospective observational study of pregnant women referred to our Maternal-Fetal Medicine Division with suspected HCMV infection from January 2007 to December 2014. Primary infection was diagnosed based on seroconversion for HCMV and/or HCMV IgM-positive and low/moderate HCMV IgG avidity, after determination of specific IgM and IgG by automated chemiluminescence immunoassay and confimatory immunoblot. Amniocentesis was performed after 20 weeks of gestation and at least 6 weeks after maternal infection. Amniotic fluid samples were subjected to a direct search for HCMV in culture and for the viral genome by real-time polymerase chain reaction (PCR). Maternal whole blood samples were analyzed by real-time PCR to determine maternal DNAemia.

With respect to fetal/neonatal outcome, newborns were classified as uninfected or infected on the basis of virus isolation and real-time PCR from urine sampled within the first two weeks of life, and in symptomatic or asymptomatic disease on the basis of fetal autopsy or pediatric follow-up.

Two hundred and forty-one pregnant women were enrolled: 32 blood samples (13.3%) were positive and 209 (86.7%) negative for the detection of HCMV-DNA. The overall rate of HCMV transmission was 23.6% (57 fetuses/newborns). Among infected cases, 8 aborted fetuses and 7 newborns (26.3%) were symptomatic and the remaining 9 fetuses and 33 newborns (73.7%) were asymptomatic. Vertical transmission occurred in 14 (43.8%) women with positive HCMV-DNAemia and in 43 (20.6%) women with negative HCMV RT-PCR (p-value 0.05).

CONCLUSION

In our experience, PCR DNA performed at the time of amniocentesis was significantly associated with congenital infection, with a 3-fold increased risk of vertical transmission. However, there was no correlation with congenital symptomatic disease at birth or at neonatal follow-up.

CMV in the fetus

HISTORICAL FINDINGS IN FETUSES WITH PROGNOSTIC MARKERS OF SYMPTOMATIC HUMAN CYTOMEGALOVIRUS INFECTION IN CORD BLOOD (ID 110)

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Abstract

Background: Clinical management of pregnant women with primary HCMV infection acquired during the first trimester of pregnancy mainly relies on HCMV detection in amniotic fluid. In our Center, cordocentesis is offered to pregnant women with HCMV-positive amniotic fluid within 21week gestation to investigate multiple parameters previously shown to be associated to poor fetal outcome.

Objectives: To retrospectively investigate histological findings in HCMV infected fetuses with virological, hematological and biochemical markers in blood predictive of symptomatic infection.

Methods: Detection of fetal infection was performed on amniotic fluid by virus culture and quantitative PCR. Fetal blood samples were examined for Beta-2 microglobulin, HCMV DNAemia, virus-specific IgM and platelet count. Infected fetuses were considered at risk of symptomatic infection when at least three out of four markers were abnormal. All women underwent ultrasound and magnetic resonance examinations. In case of elective termination of pregnancy fetal and placenta tissues underwent histological examination.

Results: Overall, 167 amniocentesis for HCMV detection were performed during the study period (Jan 2010-Feb 2016). Thirty-two women (19%) transmitted the infection to the fetus. Of these, 16 (50%) opted for cordocentesis. Prognostic markers of symptomatic congenital infection were detected in blood of 7 fetuses. Ultrasound and magnetic resonance abnormalities were also observed in two fetuses. All 7 women opted for elective termination of pregnancy. Four fetuses were available for histological examination. Cytomegalic cells in fetal brain were observed in all 4 fetuses.

Conclusions: In this limited series of cases, fetal blood prognostic markers and histological findings compatible with poor prognosis were concomitantly detected thus confirming the potential utility of cordocentesis in case of HCMV positive amniocentesis.

CMV in the fetus

VIROLOGICAL AND HISTOLOGICAL EVALUATION OF PLACENTAS FROM CHIP STUDY (ID 113)

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Abstract

The randomized placebo-controlled trial involving CMV-specific hyperimmune globulin (HIG) administration to pregnant women with primary CMV infection (CHIP study) showed a non-significant reduction from 44% to 30% of vertical transmission (NEJM 2014, 370:1316-26).

Our aim was to evaluate the virological and histological parameters on 40 term placentas collected from 20 HIG treated mothers (10 transmitters) and from 20 untreated mothers (10 transmitters) who participated to the CHIP study.

For each placenta, three full thickness sections from the central plate, through the chorionic plate to the decidua, were randomly taken. CMV-real time PCR, CMV-immunohistochemistry and ematoxylin and eosin staining were performed on each section.

Histological evaluation included 11 parameters related to tissue damage and 2 parameters related to hypoxic parenchymal compensation. A score was given to each parameter; the final score of each

parameter was the sum of the 3 sections. The slides were analysed by two independent observers blind to maternal treatment and CMV transmission. Discordant results were resolved by a third blind observer.

In both arms, placentas from non transmitter mothers were CMV negative. Comparable low levels of CMV-positive cells and viral DNA were observed in placentas of transmitter mothers in both HIG and placebo arms. Histological examination revealed a higher score of tissue damage in placentas of transmitters compare to non-transmitter mothers in both HIG (median value 18 vs. 12.5, respectively) and placebo arms (median value 17 vs. 10.5, respectively). Chronic villitis and calcifications were the parameters strongly correlated with the infection. The scores of parameters related to hypoxic parenchymal compensation were very similar in placentas of transmitter and non-transmitter mothers, irrespective of the treatment received.

HIG treatment did not reduce placental infection and histological CMV-related damage, particularly inflammation.

CMV in the newborn and beyond

LONGTERM OPHTALMOLOGIC OUTCOMES IN CONGENITAL CYTOMEGALOVIRUS (CMV) INFECTION (ID 062)

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Abstract

Background: Long term ophthalmologic and vision outcomes in children born with congenital CMV (cCMV) are poorly understood.

Methods: 237 subjects [71 symptomatic congenital CMV (ScCMV), 109 asymptomatic congenital CMV (AcCMV) with virologically proven congenital CMV infection, and 51 uninfected controls (CcCMV)], were enrolled in a longitudinal study of long term outcomes of cCMV between 1982-1995. Subjects underwent evaluations at birth, and then serial age -appropriate complete ophthalmologic evaluations by pediatric ophthalmologists, through 18 years of age.

Results: 10/77 (13%) ScCMV and none of the AcCMV and CcCMV subjects had severe visual impairment ($p<0.006$). CcCMV and AcCMV subjects had significantly better visual acuity than ScCMV subjects. Most common opthalologic sequelae in ScCMV included strabismus (23.4%), chorioretinal scars (20.8%), cortical visual impairment (14.3%), nystagmus (14.%), optic atrophy (9.1%), retinal detachment (XX%). Three ScCMV subjects had delayed visual deterioration due to possible late onset and reactivation of chorioretinitis. Severe vision impairment was associated with ScCMV status, optic atrophy chorioretinal scars, and cortical visual impairment.

Conclusions: ScCMV is associated with severe ophthalmologic sequelae. Late onset or reactivation of chorioretinitis was detected in ScCMV subjects.

CMV in the newborn and beyond

EVALUATION OF THE AUTOMATED DNA EXCTRACTION INSTRUMENT QYASIMPHONY SP® FOR CMV DNA EXTRACTION AND AMPLIFICATION ON DBS. (ID 069)

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Abstract

Congenital cytomegalovirus (CCMV) infection occurs in 0.7% of births causing long-term sensorineural hearing loss (SNHL) and neurological impairment in a significant proportion of infected infants. About 13% of infants born with symptoms and 14 % of infants asymptomatic at birth, face a significant risk of developing late sequelae (commonly SNHL). Therefore, there is a need to identify infected children for early interventions such as hearing aids, cochlear implants or speech therapy to protect speech and language development. Accurate and timely diagnosis of CCMV is essential to allow prompt recognition of sequelae and provide the opportunity for treatment. Dried bloodspots (DBS) are taken routinely after birth in many countries for bio-chemical and genetic analysis and are stored for prolonged periods of time. Numerous proof-of-concept studies have shown that CMV can be detected in DBS, however in most studies low throughput DNA extraction methods have been used.

The aim of our study was to contribute additional technical information over the possibility of DBS CMV testing in newborns. We used the automated DNA extraction instrument Qyasimphony SP® to assess the feasibility of CMV DNA detection from DBS.

We analysed a total of 100 DBS, these were obtained retrospectively from 35 infants diagnosed with CCMV by detection of HCMV DNA in plasma and from 65 infants negative for the presence CMV DNA in the plasma.

In 29 DBS obtained from the positive CMV infants (viral load ranging from 247 to 346.000 cp/ml), duplicate testing showed the presence of CMV DNA. 5 out of 6 infants with a low plasma viral load, <170 copies/ml, tested in triplicate gave at least a positive result, whereas in one sample with a low plasma viral load CMV DNA tested negative.

DBS of 65 infants negative for plasma CMV DNA were also analysed, 43 were confirmed negative. 12 samples gave a clear positive result (ranging from the detection limit of 85 cp/ml and 200cp/ml). Interestingly a plasma sample of 3 of these 12 infants was obtained during the study and CMV DNA was detected albeit at low levels. In 10 samples triplicate testing gave one positive result.

We evaluated the sensitivity of the method. 50 ul of a blood sample positive for HCMV were diluted and then spotted on filter paper in duplicate. After extraction and amplification the sensitivity of the assay was assessed as about □300 □□□cp/ml with two positive results, this data confirm that below a plasmatic viral load of 250 cps/ml false negative results can be obtained.

We demonstrated that using the automated DNA extraction instrument Qyasimphony SP® for CMV DNA extraction and amplification on DBS it is possible to correctly identify newborns with high viral loads, that are likely to be at risk for CMV infection late sequeale. However in samples with a low viral load (less than 200-300 cps/ml in the plasma) a false negative result it is possible, although infants with a low viral load are less likely to develop late sequelae. In conclusion, the automated DNA extraction instrument Qyasimphony SP® could be used for CMV DNA extraction and amplification from DBS, however improvements in DBS collection and DNA extraction are required to increase the sensitivity of the methodology.

CMV in the newborn and beyond

POSITIVE IGM AT BIRTH AND A SYMPTOMATIC CONGENITAL CYTOMEGALOVIRUS INFECTION (ID 076)

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Abstract

Background: Identification of congenital cytomegalovirus (cCMV) infection by detecting specific CMV IgM in the neonatal serum compared with viral identification in the urine and/or saliva is considered to have a low sensitivity. However, a few reports have attempted to associate the presence or absence of IgM to disease severity in neonates.

Methods: Data was collected for all infants with cCMV infection followed in our pediatric clinic, born between January 2005 and July 2015. Infant outcome after birth, symptomatic central nervous system (CNS) involvement vs. asymptomatic (no CNS involvement) was compared between infants with a positive and negative IgM test.

Results: The IgM test was obtained after birth in 199 children with cCMV. Sensitivity of positive IgM in the diagnosis of cCMV was 40.7%. The rate of symptomatic disease in those with positive IgM was statistically higher than in those who tested negative (67.7% vs. 35.8%, $p<0.001$). Odds ratio for a symptomatic disease in infants born after a maternal primary infection, diagnosed with positive IgM, compared to borderline or negative, was 3.47 (95% CI: 1.7 to 7.1). Nevertheless, a positive IgM was found in only 48.8% of symptomatic and 22.1% of asymptomatic children.

Conclusions: Our results demonstrate a low sensitivity of IgM in diagnosing cCMV. However, while a positive IgM antibody for CMV is associated with a more symptomatic disease in cases of maternal primary infection, it does not serve as a precise laboratory marker for a symptomatic disease.

CMV in the newborn and beyond

RISK FACTORS ASSOCIATED WITH HEARING LOSS AND NEUROLOGIC IMPAIRMENT IN THE SPANISH NETWORK OF INFANTS WITH CONGENITAL CYTOMEGALOVIRUS INFECTION (REDICCMV) (ID 078)

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Abstract

BACKGROUND:

We aimed to study risk factors associated with hearing loss and neurologic abnormalities at 12 months of age in the Spanish cohort of children with congenital cytomegalovirus infection (cCMV; REDICCMV).

METHODS:

A prospective multicentric study was performed from January-2011 to May-2015 in Spain. All children with confirmed cCMV were included. Hearing loss (>25dB in ABR) and neurologic abnormalities (motor impairment, microcephaly, epilepsy and neurodevelopmental delay evaluated by a pediatric neurologist) were studied at birth and at 12 months of age.

RESULTS:

297 children with cCMV from 34 hospitals were included. 229 (82.7%) children were diagnosed during the fetal or newborn period, and 111/225 (49.3%) were symptomatic at birth. Among asymptomatic infants at birth (n=61), 23.0% and 7.8% presented hearing loss and neurologic abnormalities at 12 months, respectively. Symptomatic children at birth presented higher risk of hearing loss and neurologic sequelae at 12 months of age (OR:3.2 [CI95%: 1.5-7.2] and OR:9.0 [CI95%:2.9-27.9] respectively). Blood viral load at birth was not associated with sequelae. Children with severe disease were given a longer course of antiviral treatment. In a multivariate logistic regression analysis, only hearing loss at birth was associated with hearing loss at 12 months (OR:33.2 [IC95%9.8-112.4]; p=0.0001). GPT>80 IU/L and hearing loss at birth were associated with neurologic abnormalities at 12 months (OR:7.5 [IC95%:1.0-57.0]; p=0.05 and OR:6.9 [IC95%:2.1-22.2], p=0.001 in both).

CONCLUSIONS:

In our cohort, symptomatic cCMV newborns were at high risk of sequelae at 1 year of age, which also affected one fourth of asymptomatic patients at birth. Hearing loss at birth was associated with both hearing loss and neurologic impairment at 1 year of age; neonatal hepatitis was also a risk factor for neurologic sequelae at 12 months of age.

CMV in the newborn and beyond

WHAT IS THE DIAGNOSTIC, THERAPEUTIC AND FOLLOW-UP MANAGEMENT OF NEONATAL AND PERINATAL CYTOMEGALOVIRUS INFECTION IN MARSEILLE? (ID 088)

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Abstract

Introduction:

There isn't consensus of management of pregnant women or new-borns and infants suspected or known to have cytomegalovirus (CMV) congenital or perinatal infection in France [1-4].

We present here the results of a descriptive, retrospective, observational study. The objectives were to evaluate the diagnostic, therapeutic and the follow-up management of women, new-borns and infants for whom congenital or perinatal CMV infection was certified.

Material and method:

We analyzed the medical record of fœtus, new-borns or infants for whom a CMV PCR was positive on amnios fluid, urine, fresh blood or dry blood, in the microbiological laboratory of Marseille University Hospital from May 2013 to February 2016.

Results:

CMV congenital infections were diagnosed for 4 fetus and 29 new-borns or infants.

There were 9 antenatal diagnoses. Delay between birth and diagnosis was inferior to one month in 10 cases, between 1 and 6 months for 12 infants, between 6 and 24 months for 2 of them. The department where the diagnosis was done was gynecological-obstetrical department in 9 cases, Maternity and neonatal department in 17 cases, intensive care unit in 3 cases, neurological department in 2 cases, ORL department in 1 case, hepato-gastro-enterology in 1 case. CMV serology with Immunoglobulin avidity was done for 21 pregnant women. 10/32 cases were known to be secondary to pregnant women's CMV seroconversions, 7/32 secondary to pregnant women's CMV reactivations or reinfections and 4/32 due to post-natal contaminations because of breast-feeding. On 33 pregnant women 9 were symptomatic. In 2 cases there was VIH co-infection, in 1 case there was HSV co-infection. 8 pregnant women benefited of an antenatal fetal IRM, 3 of them were abnormal. PCR CMV on amnios fluid were performed in 6 cases (all positive), PCR CMV on placenta in 5 cases (all positive), PCR CMV on pediatric patients' fresh blood in 26 cases (23 positive, 2 negative, 1 undefined), PCR CMV on fresh urines in 24 cases (all positive), PCR CMV on saliva in 7 cases (5 positive, 2 negative), PCR on stool samples in 4 cases (1 positive, 3 negative), PCR CMV on cerebrospinal fluid in 5 cases (1 positive, 4 negative) and CMV serology in 14 pediatric patients (all positive). 15 pediatric patients had a blood viral load superior to 10 000 copies per mL. At diagnosis, 6 pediatric patients had intrauterine growth delay, 12 had hematological abnormalities, 16 had neurological abnormalities, among them 14 had clinical neurologic symptoms, 3 had ORL symptoms (surdity), 4 had hepatobiliary and digestive symptoms, and 4 had pneumological signs. 1 pregnant woman has benefited of antenatal treatment. 11 pediatric patients has benefited of a postnatal treatment, 9 by valganciclovir, among them 6 were included in CYMEPEDIA research protocol, 1 was treated by ganciclovir and for one this data is unavailable. There were 4 medical pregnancy interruptions and during the follow-up of infant, 6 pediatric patients died, 23 were symptomatic, 6 had serious sequeleas during follow-up, 8 recovered health. We noticed that 16 infants were premature baby, which can introduce a bias concerning neurological sequelaes.

Conclusion:

This study shows that there is a big disparity in management of patient with congenital or neonatal CMV infection in Marseille. This is consistent with data published in literature. This is the witness of a lack of consensus in this field. Heterogeneity of management seems to be conditioned by seriousness of symptom presenting by infant. Nowadays intravenous ganciclovir (CYMEVAN®) [5] and oral valganciclovir (ROVALCYTE®) [6] seem to be well support by new-borns and infants [7]

and seem to have a good effectiveness to avoid neurological or auditive complications if started early. [3, 5-8] As the viral neonatal charge seems to be a good complication risk's marker, [9, 10] it could be interesting to test every new-born in order to treat them in case of CMV infection with high level of viral charge. [11, 12] For example we could imagine to do a systematic CMV PCR on urine sample [1-4], dry blood spot filter paper [13] or saliva [14] in the days following birth.

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CMV in the newborn and beyond

CMV GENOTYPES IN NEONATES AND INFANTS BELOW 1 YEAR OF AGE. (ID 093)

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Abstract

CMV genotypes might influence the impact of the CMV infection on the developing fetus and very likely the developing infant in the first year of the post-natal development. So far, only limited number of studies among these infants combined the glykoprotein B (gB), glykoprotein H (gH) and UL144 typing. Aim of our study was therefore to find out chat genotypes could be observed in the biggest hospital in the Czech Republic.

Between 2002 and 2014, we have tested 592 biological samples from 94 children bellow 1 year of age for presence of CMV. In total, we have tested 456 whole blood, 94 urine samples and 42 other biological samles (most frequently CSF). Detection was performed by quantitative PCR technique and results were normalised to 10,000 human genome equivalents assessed by quantification of albumin gene in the whole blood (normalised viral copies – NVCs) and different tissue samples and in CMV copies in 1 ml of the whole urine. Retrospective CMV genotyping of gB1-4, gH1 and gH2 was performed according the published studies by real-time PCR detection and UL144 sequencing.

CMV was detected in 317 samples from 96 children. In 13 neonates, we detected CMV until two weeks of age in neonates proving the congenital CMV infection. Median of detected CMV load in whole peripheral blood was 6.42 CMV NVCs (range 2.24-376.65 NVCs) and median of 7.5x10e3/ml CMV copies/ml of urine (range 5x10e2-8.1x10e6). In 11 children with congenital CMV, clinical assessment was performed (with median 6.25 years of age (2.87-12.49 yrs.) and in 2 children, sensoneural hearing loss was detected.

Glycoprotein B or gH only was detected in only 9 infants. Total genotyping with both gBgH was detected in 68 children (gB1gH1 in 13, gB1gH2 in 14, gB2gH1 in 4, gB2gH2 in 9, gB3gH1 in 6, gB3gH2 in 3, gB4gH1 in 7, gB4gH2 in 3 and different mixed infection were detected in 9 children (one neonate)). Good UL144 sequence was obtained so far from 44 children and sequence was determined as UL144 type A in 12 children, B in 18 children and C in 8 children, AB genotype in 6.

Complexity of human CMV infection and its different impacts remains one of the possible biological enigmas of this infection and further studies aimed different CMV genotype and tissue tropism should help in the assessment of the different risks and necessity of the virostatic treatment in particular patients.

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CMV in the newborn and beyond

CONGENIT CMV IN YOUNG INFANTS (ID 097)

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Abstract

Introduction

Cytomegalovirus (CMV) is the most frequent cause of neurosensorial hearing loss and neurodevelopmental delay in young infants. At birth, most of neonates are asymptomatic, only 10% of them have clinical features. Since no data are reported from LatinaAmerica on CMV in young infants, we sought to review history charts of patients with confirmed CMV infection in infants in our Institution.

Results

Twenty-three patients with congenital CMV were evaluated and care in between June 2010 and February 2016. Median age at diagnosis: 19 days

Only 56,5 % (13) of cases were diagnosed in the neonatal period, 30,5% (7) between 1-6 months (mo) and 13% (3) after 6 mo of age.. Eighty-three percent of cases had symptoms at birth. Median maternal age was 26 years old (r 16-40) and 55% of them had symptoms. Prenatal ultrasound detected anomalies in 20%. The most frequent clinical features at diagnosis were neurological symptoms in 69,5% (17/23), microcephaly 43,4% (10/23), jaundice 43,4% (10/23) , hearing loss 39% (9/23), ophtalmologic alterations in 39% (9/23), petechiae in 17,4% (6/23) y pneumonitis 4,3% (1/23). The most frequent biochemistry abnormalities were: increased liver enzymes in 43,4%, anemia 30,4% and thrombocytopenia in 26%.

Cerebral ultrasound or CT scan were done in 20/23 (%) patients, abnormalities were detected in 85% (17/20) (calcifications 65% and ventriculomegaly in 60%). CNS MRI was done in 10/23 patients, pathologic changes were seen in 80% of cases (60% white matter abnormalities, 20% cortical compromise).

Diagnosis was confirmed by CMV-PCR in 91,3% in blood and/or urine and in 8.7% by CMV-PCR on Guthrie card.

Mean CMV viral load in blood was 6157 cp/ml (r 0-14.329) at diagnosis for 5 patients 1 month.

Treatment: 15/23 pts were treated with ganciclovir IV or Valganciclovir. 9/15: pts < 1 month and 6/15 > 1 mo. Ganciclovir 6 weeks and 7 were shifted to Valganciclovir (treatment course: 40 days-11mo). All patients normalized the hematological and liver tests.

Adverse events were detected in 3/15 (20 %), all were hematological abnormalities.

All children with hearing loss (9/9) had abnormal neuroimaging abnormalities(100%), 78% (7/9) had mental delay and 44% (4/9) thrombocytopenia vs 57.1% (8/14), 64.2 % (9/14), 21% (3/14) respectively in children with normal hearing

Conclusions

The high incidence of symptomatic pts could be reflected that our Institution is a reference center in Argentina.

Abnormal brain images at birth are correlated with risk neurodevelopmental delay and hearing loss, which also correlates with thrombocytopenia at birth. Of patients treated 20%had adverse events. All normalized biochemical alterations, but we can´t make conclusions about the other parameters because it is not a randomized study and there are different treatment duration, further studies are need in Argentina.

CMV in the newborn and beyond

EVALUATION OF CELLULOSE PADS AS A NEW METHOD TO DETECT CYTOMEGALOVIRUS DNA IN THE URINE OF NEONATES (ID 098)

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Abstract

Background: Congenital cytomegalovirus (CMV) infection occurs in 0.2 to 2.2% of neonates. A significant proportion of infected infants do not present with symptoms and might only be detected by routine screening methods. Various methods of CMV DNA detection has been investigated in the past. In our study, a new method using cellulose pads for urine collection was evaluated in an experimental setting and in clinical practice.

Methods: Cellulose pads regularly used for maternity care (Samu Woechnerinnen Vorlagen, Hartmann AG, Germany) were used for all experiments including urine collection in neonates. We systematically tested the effect of storage duration of the pad after exposure with CMV positive urine, meconium contamination and specimen handling on the CMV viral load and the detection rate from urine of neonates with congenital CMV. Further, the method was tested as a screening method in clinical practice in a cohort of 500 neonates.

Results: Following exposure of the urine pad with CMV positive urine, the viral load decreased after 15 minutes, 12 hours, 24 hours and 7 days of storing in a plastic bag, to 63.2%, 42.1%, 31.6% and 9.3% of the baseline value. The detection rate was 100 % after 7 days. Contamination of urine with meconium resulted in a further reduction of the viral load. The detection rate in exposed urine pads letting dried for 7 days was 93.3%. In a clinical cohort of 500 neonates the prevalence was 0.4%. Urine collection from the urine pads was successful in 73.6% (368/500 infants) by the first attempt and in 26.4% (132/500 infants) by the second attempt.

Conclusions: In infants urine collection using cellulose pads seems feasible regardless of a reduction of the CMV viral load due exposure to the pad itself or to meconium contamination. Drying of the exposed urine pad should be avoided.

CMV in the newborn and beyond

EVALUATION OF CMV-SPECIFIC CD8+ T-CELL RESPONSES IN NEONATES WITH CONGENITAL CMV INFECTION USING QuantiFERON®-CMV ASSAY: PRELIMINARY RESULTS (ID 099)

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Abstract

BACKGROUND: Cytomegalovirus (CMV)-specific cell-mediated immunity (CMI) plays a crucial role in the control of CMV replication. A failure in CMV-specific CMI is associated with higher peak viral loads, higher incidence of recurrent or complicated CMV reactivation in transplant recipients, as well in critically ill patients. Effector functions of CMV-specific T-cells have been successful assessed in these settings using QuantiFERON®-CMV assay, that evaluates the amount of interferon-gamma (IFN-γ) secreted by CMV-specific CD8+ T lymphocytes upon in vitro stimulation of whole blood with viral antigens. The aim of this study is to evaluate the usefulness of the secretion of IFN-γ by CMV-specific CD8+ T-cells using QuantiFERON®-CMV (Cellestis-Qiagen) assay in neonates with congenital CMV infection and whether a lack of CMV-specific CMI correlates with the clinical course.

METHODS: Term neonates with congenital CMV infection, diagnosed by CMV isolation in urine in the first two weeks of life, were included. A blood sample for QuantiFERON®-CMV assay was obtained within the end of the second week of life; a second sample was obtained within the end of the second month of life. For the QuantiFERON®-CMV test 1 mL of heparinized whole blood was collected into each of the three QuantiFERON®-CMV tubes: CMV peptide pool ("CMV"), negative control ("nil") and positive control containing phytohemagglutinin ("mitogen"). The interpretation of IFN-γ response was as follows: positive if [CMV-nil]≥0.2 IU/mL; negative if [CMV-nil] <0.2 IU/mL and [mitogen-nil]≥0.5 IU/mL; indeterminate if [CMV-nil]<0.2 IU/mL and [mitogen-nil]<0.5 IU/mL. Specifically, a positive QuantiFERON®-CMV result identifies a patient with detectable CMV-specific CMI; a negative result identifies a patient without CMV-specific CMI but with general T-cell responsiveness; results are reported as indeterminate in not responders to both CMV and

mitogen stimulation identifying patients without any CMI. Data regarding CMV infection in pregnancy and clinical and instrumental evaluations in the first months to detect symptoms/signs of infection were analyzed.

RESULTS: Nine term infants with congenital CMV infection were included. The mean age at blood samples collection was 9 days for the first evaluation (range 5-15days), and 46 days (range 33-62days) for the second evaluation. Five out of 9 infants (55%) were interpreted as QuantiFERON®-CMV positive at first sample, and this result was confirmed at the second evaluation. Quantitatively all these samples had an IFN-γ level [CMV-nil] ≥ 1 IU/mL. All these infants were born to mothers with a primary CMV infection during the first trimester in one case, during the second trimester in two cases and during the third trimester in two cases. None of these infants showed signs of symptomatic CMV infection. Four out of 9 infants (45%) were interpreted as QuantiFERON®-CMV negative at first sample. Two out of these 4 infants (50%) were born to mothers with a primary CMV infection occurred during the first trimester of pregnancy; both these infants resulted QuantiFERON®-CMV negative also at the second evaluation, and both were defined as symptomatic (sensorineural hearing loss in one case and cerebral abnormalities in the other one) and were treated with oral valganciclovir. One out of 4 infants (25%) was born to a mother with a non-primary CMV infection during the third trimester of pregnancy: this infant had a positive QuantiFERON®-CMV result at second evaluation, meaning the development of a CMV-specific cell-mediated response. This infant remained asymptomatic. The remaining infected infant (25%) was born to a mother with a primary CMV infection during the third trimester of pregnancy; this infant had a confirmed negative QuantiFERON®-CMV result at second evaluation and remained asymptomatic.

CONCLUSIONS: Our preliminary results seem to suggest that QuantiFERON®-CMV assay could be useful in the evaluation of infants with congenital CMV infection in order to early recognize the high risk of development of sequelae. The CMV-specific CD8+ T-cell responses do not seem to be influenced by the time of maternal infection. The presence of strong CMV-specific CD8+ T-cell responses during the first two weeks of life seems to correlate with the maintenance of the anti-viral specific responses and with an absence of symptomatic infection at birth and during the follow-up. Instead, infants with a lack of CMV-specific CD8+ T-cell responses during the first two weeks of life should be retested in the second month of life to verify the development of a CMV-specific CMI, as a persistent negative QuantiFERON®-CMV result seems to correlate with an higher risk of symptomatic CMV infection. However, given the small sample size, further studies are needed to better understand the performance of QuantiFERON®-CMV assay in congenital CMV infected infants.

Table / Image

Table. QuantiFERON®-CMV assay in relation to maternal infection and clinical course

Patient	Maternal infection	1° QuantiFERON®-CMV assay	2° QuantiFERON®-CMV assay	Symptoms/signs of infection	Last follow-up evaluation
1	Primary, 1° trimester	POSITIVE (8.12 IU/ml)	POSITIVE	no	15 months
2	Primary, 2° trimester	POSITIVE (>10 IU/ml)	POSITIVE	no	12 months
3	Primary, 2° trimester	POSITIVE (6.2 IU/ml)	POSITIVE	no	34 months
4	Primary, 3° trimester	POSITIVE (1 IU/ml)	POSITIVE	no	36 months
5	Primary, 3° trimester	POSITIVE (>10 IU/ml)	POSITIVE	no	20 months
6	Non-primary, 3° trimester	NEGATIVE	POSITIVE	no	12 months
7	Primary, 1° trimester	NEGATIVE	NEGATIVE	Sensorineural hearing loss	36 months
8	Primary, 1° trimester	NEGATIVE	NEGATIVE	Cerebral abnormalities	20 months
9	Primary, 3° trimester	NEGATIVE	NEGATIVE	no	28 months

CMV in the newborn and beyond

OBSERVATIONAL STUDY OF CHILDREN WITH CMV INFECTION BORN IN GREECE. THE ROLE OF CMV-HIG AND ANTIVIRAL TREATMENT. (ID 101)

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Abstract

Congenital cytomegalovirus infection (cCMV) represents the most common congenital infection. Although most newborns are asymptomatic, cCMV is associated with severe sequelae including sensoneural hearing loss (SNHL) and neurological disabilities. There is an ongoing debate in literature whether prospective screening of CMV-seronegative pregnant women versus universal neonatal screening is most effective strategy to minimize sequelae in newborns with cCMV. Furthermore, the role of maternal primary infection versus reactivation during pregnancy remains controversial. To answer these questions, one needs to consider whether CMV-HIG administration to pregnant women with CMV-infection decreases transmission and/or disease severity, to estimate the CMV-seropositivity levels among women of childbearing age and appraise the effectiveness of antiviral treatment of the newborn with cCMV. To explore this complex dilemma we retrospectively analysed data from 47 infants with cCMV followed in our center. Table below depicts cohort characteristics.

As expected women with primary infection were more likely to receive CMV-HIG (p=0.004). Among the 33 children followed for more than 24 months, 25 children (75.8%) had favorable outcome with no SNHL or neurologic sequelae. However, this could not be attributed to maternal CMV-HIG therapy, antiviral treatment of the newborn or both due to small numbers. Most importantly, since this is a retrospective study, treatment selection bias applies.

In conclusion, the most important factor associated with outcome was gestational age at the time of intrauterine CMV infection while larger cohorts are needed to explore the role of therapeutic maneuvers.

Table / Image

CMV maternal status	Primary CMV infection	Re-infection reactivation	Total
No of cCMV children	35	12	47
CMV-HIG treatment	31	3	34
Symptomatic newborns*	15	4	19
Babies treated with antivirals	14	4	18

* including asymptomatic babies with abnormal head US/MRI

CMV in the newborn and beyond

LATE-ONSET SENSORINEURAL HEARING LOSS IN INFANTS WITH CONGENITAL CYTOMEGALOVIRUS INFECTION AND PROLONGED ANTIVIRAL TREATMENT. (ID 103)

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Abstract

Background

Late-onset sensorineural hearing loss (SNHL) in congenital cytomegalovirus (cCMV) infection is rarely described in long-term treated patients. We report 4 children who deveoped late-onset NSHL after finishing prolonged antiviral treatment.

Case reports

Since 2009, 27 infants with cCMV infection received antiviral treatment for at least 6 months and have been followed up for more than 2 years. Four (14.8%) developed late-onset SNHL. All were symptomatic at birth; three had abnormal central nervous system ultrasound (US) and one intrauterine growth restriction and unilateral hearing loss. All received ganciclovir (14-42 days) and valganciclovir (4.5-6 months). Hearing was assessed every 6-12 months by brainstem auditory evoked response (BAER). Patient 1: normal BAER at birth in left ear, no response in right ear (>90dB). At 2.4 years-of-age, no response bilaterally. Patient 2: normal BAER at birth. At 2.6 years-of-age, right ear responses at 60dB, no response in left ear (>90dB), at 3.2 years-of-age: no response bilaterally. Patient 3: BAER at birth 20 dB left ear, normal right ear. At 3 years 60 dB left ear, 20 dB right ear. At 6 years 60 dB left ear, 40 dB right ear. Patient 4: BAER at birth: normal left ear, 40-60 dB right ear. At 6 years 80 dB in right ear, and one year later drop of 35dB in left ear in high frequencies. Patients 1 and 2 had cochlear implants placed and the others use hearing aids.

Discussion

Late-onset NSHL can appear in congenital CMV infants after a prolonged course of antiviral treatment, even years after diagnosis. We recommend prolonged follow-up to identify these cases and larger studies to identify high-risk patients.

CMV in the newborn and beyond

CYTOMEGALOVIRUS REAL TIME POLYMERASE CHAIN REACTION IN DRIED BLOOD SPOTS: CAN WE REALLY RULE CONGENITAL INFECTION OUT?(ID 104)

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Abstract

Brief Introduction: The detection of cytomegalovirus (CMV) DNA by real time polymerase chain reaction (rt-PCR) in neonatal dried blood spots (DBS) collected for metabolic screening has been assessed in many studies for the retrospective diagnosis of congenital CMV (cCMV). A previous small study performed by our group in 2013 showed that sensitivity may be in the lower range (50%) when compared to data previously reported elsewhere (34-100%). The aim of this study was to assess the effectiveness of this technique in our country in a larger Spanish series.

Patients and methods: multicentric ambispective study that included patients with confirmed cCMV (defined by CMV detection in sterile sites before 14 days of life) included in the Spanish Registry of cCMV patients (RedicCMV, currently 332 patients recruited) and born between January 2007 and January 2016. The assessment of CMV DNA in DBS was made by using rt-PCR (Artus CMV PCR kits CE®;Qiagen Inc., Valencia, CA, USA). The following variables were collected: gender, maternal symptoms during pregnancy, time elapsed between DBS collection and processing, plasma viral load (PVL) at birth, symptoms at birth, hearing loss at birth and during follow up, neurodevelopment and antiviral therapy. Children in whom cCMV had definitively been ruled out (negative urine PCR CMV at birth) were used as negative controls.

Results: One-hundred and five children with confirmed cCMV from 10 hospitals and sixty negative controls were included. From 105 patients with cCMV, 103 DBS were finally processed. Fifty-eight of them tested positive: sensitivity 0,5631 (CI: 0,4668 to 0,6549). Lower PVL at birth was associated with a negative result in DBS rt-PCR (p=0.017). No other variables, except for thrombocytopenia that showed marginal statistical significance (p=0.055), were associated with DBS rt-PCR negative results.

Specificity was 0,9667 (CI: 0,8864 to 0,9908) (58 out of 60 had negative results). Positive likehood ratio was 16,893 (CI: 4,279 to 66,693), but negative likehood ratio was low: 0,452 (CI: 0,361 to 0,566)

Conclusions: The sensitivity of rt-PCR in DBS in our cohort was lower than previously reported. Although positive likehood ratio value is quite good, a negative DBS result does not fully predict the absence of cCMV infection, especially in patients with low PVL at birth.

CMV in the newborn and beyond

BRAIN DAMAGE AND NEURODEVELOPMENTAL OUTCOME IN CHILDREN WITH SYMPTOMATIC AND ASYMPTOMATIC CONGENITAL CMV INFECTION (ID 107)

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Abstract

Introduction

Congenital cytomegalovirus (CMV) infection is the most common viral intrauterine infection and the leading infectious cause of neurodevelopmental disabilities. In symptomatic disease the brain is the most affected organ as the virus affects fetal neuronal stem cells, which are particularly susceptible to infection and differentiated neuronal cells (1). Besides this direct influence virus also affects placental cells, leading to placental insufficiency and subsequent indirect fetal brain damage. Majority of children with symptomatic disease and also some of those, who are asymptomatic at birth, develop long-term neurodevelopmental disabilities such as motor developmental delay, intellectual disability, sensorineural hearing loss (SNHL) and visual impairment.

Patients and methods

Brain US and MRI findings and neurodevelopmental outcome of children with congenital CMV infection, born after 1996 and followed at University Medical Centre Ljubljana, were retrospectively analyzed.

Results

Nineteen children with congenital CMV infection were found; 11 (58 %) boys and 8 (42 %)girls; their average age was 7.3 years (range 1 to 17 years). Fourteen (74 %) had symptomatic and 5 (26 %) had asymptomatic disease. Five (36 %) symptomatic newborns were treated with parenteral gancyclovir. There was no fatal outcome. Brain US and MRI findings and neurodevelopmental outcome are presented in Table 1 and Table 2.

Discussion

The most frequent type of brain damage in the group of symptomatic children were hypoplasia or aplasia of corpus callosum, periventricular calcinations, hydrocephalus and cysts in periventricular

white matter, which were also described in similar studies (2, 3). On the other hand, the degree and the spectrum of brain damage in the group of asymptomatic patients were low and limited to ultrasonographic finding of lenticulotriate vasculopathy, which is a frequent, but nonspecific finding.

In the group of symptomatic children the most common neurodevelopmental disabilities were epilepsy, cerebral palsy, motor developmental delay, intellectual disability, speech disorder and SNHL. The frequency and the degree of abnormal neurological outcome corresponded to the degree and the intensity of abnormal brain US and MRI findings. The incidence of SNHL in symptomatic patients was twice as frequent as in asymptomatic children, which is consistent with the data in the literature (4).

There was an evident difference in the occurrence of neurodevelopmental disabilities between the group of symptomatic patients and newborns, which were asymptomatic at birth. However, abnormal neurodevelopmental outcome, particularly SNHL, learning disabilities and behavioral disorders, appeared also in the group of newborns, which were asymptomatic at birth. These children often develop variable or stable form of SNHL (4, 5), which was also present in this group of children. Two children had mild SNHL at the age of one month, which subsequently improved. One child, who was asymptomatic at birth, developed bilateral and permanent SNHL at the age of one year.

All treated children developed mild or moderate (but not severe) degree of neurodevelopmental disabilities, mainly motor developmental delay, intellectual disability and SNHL. Two patients, who were not treated and had mild and moderate symptomatic disease, both developed SNHL.

Conclusion

Congenital CMV infection can cause, particularly in symptomatic patients, a wide spectrum of brain damage, which correlate with long-term neurodevelopmental outcome. Findings, which are present at birth, may deteriorate further during life.

Table 1: Brain US and MRI findings in 19 children with symptomatic and asymptomatic congenital cytomegalovirus (CMV) infection

Brain US and MRI findings	*Symptomatic congenital CMV infection (n=14)*	*Asymptomatic congenital CMV infection (n=5)*
Hypoplasia or aplasia of corpus callosum	5 (36 %)	0
Periventricular calcinations	4 (29 %)	0
Hydrocephalus	4 (29 %)	0
White matter cysts	4 (29 %)	0
Lenticulotriate vasculopathy	2 (14 %)	1 (20 %)
Cerebral atrophy	2 (14 %)	0
Developmental changes of cerebral cortex (polymicrogiria, lisencephally)	2 (14 %)	0
Cerebellar atrophy	1 (7 %)	0

Table 2: Neurodevelopmental outcome of 19 children with congenital cytomegalovirus (CMV) infection

Neurodevelopmental outcome	*Symptomatic congenital CMV infection (n=14)*	*Asymptomatic congenital CMV infection(n=5)*
Epilepsy	10 (71 %)	0 (0 %)
Cerebral palsy	9 (64 %)	0 (0 %)
Motor developmental delay	9 (64 %)	1 (20 %)
Intellectual disability	6 (43 %)	0 (0 %)
Speech disorder	6 (43 %)	0 (0 %)
Sensorineural hearing loss	5 (36 %)	1 (20 %)
Microcephaly	4 (29 %)	0 (0 %)
Visual impairment	1 (7 %)	0 (0 %)
Learning disabilities, behavioral disorders	1 (7 %)	0 (0 %)
Autism spectrum disorders	1 (7 %)	0 (0 %)

CMV in the newborn and beyond

PROGNOSTIC VALUE OF CYTOMEGALOVIRUS DNA IN CEREBROSPINAL FLUID OF NEONATES WITH CONGENITAL INFECTION (ID 111)

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Abstract

Brief introduction

Cytomegalovirus (CMV) DNA detection in cerebrospinal fluid (CSF) by polymerase chain reaction (PCR) has been previously linked with poor neurodevelopmental outcomes in congenitally infected neonates. We compared patients with positive CMV-PCR in CSF with those with negative results in a large Spanish birth cohort.

Methods

An observational multicenter study (29 hospitals) was performed using Spanish Congenital Cytomegalovirus Infection Database (REDICCMV; http://www.cmvcongenito.es). Patients in whom CMV-PCR in CSF was performed were evaluated; lumbar punctures (LP) were performed as per physician decision in each center. Those with immunodeficiency, maternal immunodeficiency, other congenital infections and traumatic LP were excluded.

Results

From 311 neonates included in the database, 173 had a LP performed. A total of 136 patients met inclusion criteria: 21 (15.4%) with positive CMV-PCR in CSF and 115 (84.6%) with negative results. Seventeen patients with positive CMV-PCR in CSF were symptomatic at birth (81%), compared with 52.2% of infants in the negative group (OR: 3.86; 95% CI 1.28-14.1; p=0.01). There were only 4 asymptomatic newborns with positive CSF PCR (6.8%). Microcephaly (9.5% vs. 8.7%), brain ultrasound abnormalities (52.4% vs. 34.8%), brain MRI abnormalities (73.3% vs. 63%), hearing loss in any ear (57.1% vs. 31.3%) and neurological abnormalities at 6 months of age (42.9% vs. 23%) were more frequent in the positive CSF-PCR group, although differences were not statistically significant. Results were similar regarding plasma viral load, with higher values in the positive PCR group (median 21000 copies/ml vs. 5750; p=0.98).

Conclusions

Positive CMV-PCR result in CSF is associated with symptomatic congenital CMV infection at birth, while in asymptomatic patients occurs rarely as an isolated finding. No differences in neuroimaging studies, plasma viral load or outcome could be demonstrated between CMV-PCR in CSF positive and negative groups. Our results suggest that CMV detection in CSF lacks prognostic value in infants with congenital CMV infection.

Growing up with congenital CMV

Cerebral palsy in children with symptomatic cytomegalovirus infection - clinical features and neuroimaging findings (ID 063)

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Abstract

Background: Congenital cytomegalovirus infection (cCMV) is the most common infectious cause of neurodevelopmental disorders in childhood, including cerebral palsy (CP). In Australian CP register children with cCMV infection accounted for 1.5% of all CP cases with prevalence of 2.5/100 000 live births. Our aim is to present clinical features and neuroimaging findings in children with symptomatic cCMV infection. Study comprised 18 children with proven cCMV infection who were diagnosed as having CP. Classification of CP and associated impairments was performed according to proposal of Surveillance of Cerebral Palsy in Europe. At the time of diagnosis and classification children were aged between 4 and 16 years. Only one child was preterm, while 17 children were termborn. Neuroimaging study has been performed in all children, initially CT in older children, and later MRI in all.

Results: 15/18 patients had bilateral spastic (BS) CP, two had dyskinetic and one child ataxic CP. Five children with BS CP had good walking abilities and six were wheel chair dependent. In all patients BS CP was accompanied by moderate to severe mental retardation (MR), in 9 by epilepsy, in 7 by sensorineuronal deafness (SND), in another 7 by chorioretinitis and by autistic spectrum disorder (ASD) in one. Dyskinetic CP was in both patients of most severe grade, GMFCS V, accompanied by severe MR, SND, chorioretinitis and ASD. The only child with ataxic CP, had GMFCS I, but had severe mental retardation. Microcephaly was present in 15/18 children. Brain malformations were the predominant imaging pattern in 11/18 of children with cCMV and CP, the most common polimicrogyria and pachygyria, in four children each, lissencephaly in two and shizencephaly in one child. Migration disturbances were accompanied by cerebellar hypoplasia in four and leukoecephalopahy in four children. Isolated leukoencephalopathy was present in 6/18 cases, diffuse with bilateral temporal cysts in two. One child had marked brain atrophy and hypoplastic corpus callosum. Calcifications were present in 12/18 children.

Conclusion: Cerebral palsy following symptomatic cCMV infection is in most cases a severe condition. Associated impairments are overrepresented and often severe (particularly hearing). It is more often in females and term born. Brain malformations are the most common neuroimaging pattern.

Growing up with congenital CMV

PROGRESSIVE SENSORINEURAL HEARING LOSS IN A GIRL WITH CONGENITAL CYTOMEGALOVIRUS INFECTION – CASE REPORT (ID 106)

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Abstract

INTRODUCTION: Congenital cytomegalovirus infection (CMV) affects 1% of the live-born infants. Around 10% of children with conatal infection at birth have severe disease that includes intrauterine growth restriction (IUGR), jaundice, purpura, hepatosplenomegaly, microcephaly, intracerebral calcifications, and retinitis.The most common permanent consequence of the CMV infection - sensorineural hearing loss, can possibly develop even several years after the infection.

PURPOSE of this case report is to describe clinical course of the congenital CMV infection in a girl with a special focus on sensorineural hearing loss.

CASE REPORT: We represent a fifteen-year-old girl born from the first pregnancy at 38th week of gestation. In early newborn period, IUGR, jaundice, the signs of intrauterine and etiologically unproven infection were noticed. Brain US showed granulation of periventricular white matter. Universal neonatal hearing screening at that time was unavailable in Croatia. Due to delay in motor development at the age of 2,5 years, she was clinically evaluated. The girl was included in habilitation. At the age of 5,5 years hearing impairment was noted. Diagnostics (audiometry, ABR) concluded the complete sensorineural hearing loss on the left side.CMV serology showed positive IgG and negative IgM antibodies result. Brain MRI showed hyperintense lesions in periventricular white matter, the largest by trigonum of the left lateral ventricle. Metabolic analysis excluded leucodystrophy. At the age of 9 she underwent adenectomy due to recurrent otitis followed by hearing deterioration. Progressive sensorineural hearing loss extended on the right ear. MR brain imaging showed no progression of the lesions. The advancement in postnatal diagnostics PCR on CMV from a dried blood drop (Guthrie card) enabled etiological diagnostics. Hearing aid was provided for the right ear. She had adequately developed speech and average level of intellectual functioning.

CONCLUSION: Congenital CMV infection in 15% of the diseased leaves permanent neurological damage, most commonly sensorineural hearing loss and learning difficulties. Gestational age during period of infection determines patological changes in the brain. It is imperative to recognize the disease on time in order for patient to undergo of adequate treatment. Based on clinical picture, MR brain imaging, serology and subsequent retrograde PCR diagnostics, it was concluded that etiological diagnosis was congenital CMV infection.

Growing up with congenital CMV

THE IMPACT OF CARING FOR CHILDREN BORN WITH SYMPTOMATIC CONGENITAL CYTOMEGALOVIRUS INFECTION ON THEIR FAMILIES (ID 116)

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Abstract

Background: Parental stress and family functioning in relation to caring for a child born with symptomatic congenital cytomegalovirus (SCC) have not been systematically studied. Aim: To examine parents' perceptions on family impact of SCC and its sequelae.

Methods: The Family Impact Questionnaire (FIQ; Donenberg & Baker 1993) was administered to the parents of 22 children born with SCC (mean age 9.1±5.1y, range 31m-18y). Moderate/severe disability was defined as: cerebral palsy with GMFCS level ≥II; Bayley III cognitive, language or motor scores ≤84 or global IQ ≤70; epilepsy; visual deficit or behavioral/emotional disorders according to the CBCL DSM-oriented scales.

Results: The FIQ results are presented in the Table.

Cognitive impairment was associated with a negative impact on finance (P=0.007), whereas behavioral disorders were associated with a negative impact on marriage (P=0.040). Both cognitive deficit and behavioral problems were independently associated with a negative impact on family social life (P<0.05).

Conclusions: The FIQ is able to identify areas of family functioning affected by SCC and its sequelae. This information is useful to establish individualized multidisciplinary support. The potential impact of behavioral disorders in children without motor/cognitive disabilities should not be underestimated.

Table / Image

Table. FIQ results in 22 families of children with SCC, absent/mild disability vs moderate/severe disability

Areas of family functioning	Absent/mild disability (N=10)	Moderate/severe disability (N=12)	*P*
	Score (mean±SD)/maximum potential score*		
Feelings toward the child	10.1±6.9/45	16.7±12.7/45	0.057
Social life	0.7±1.2/30	5.4±4.2/30	0.022
Finance	0.8±1.6/21	9.9±5.5/21	0.001
Marriage§	N=9 2.9±3.3/21	N=8 9.5±7.2/21	0.015
Siblings¶	N=4 3.2±1.2/27	N=10 7.1±3.6/27	0.050

*Higher scores indicate more negative impact

N is specified for separated (§) and one-child families (¶)

New therapeutic approaches

NEW INSIGHT INTO STRATEGIES EMPLOYED BY HUMAN CYTOMEGALOVIRUS IN IMMUNOMODULATION (ID 060)

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Abstract

The selective pressure that is exerted by the immune system has shaped the pathogen evolution, in turn, the immune system has evolved to protect against the many pathogens that are encountered throughout the lifetime of an individual. This co-evolutionary relationship between host and pathogen is particularly clear for viruses that establish persistent infections, such as human herpesviruses. Human cytomegalovirus (HCMV, a β-herpesvirus), is a large double-stranded DNA virus that causes a lifelong, persistent/latent infection in about 50-80% of the US population. While HCMV infection is largely asymptomatic in healthy persons, it can induce serious disease in those with naïve or compromised immunity, and the high incidence of congenital infection has spurred a strong initiative for vaccine development. Primary clinical isolates carry at least 19 additional genes within the UL/b' genomic region that have been lost in several commonly used HCMV strains, with several of them targeting signaling by the TNF superfamily. Here, we present that the immunomodulatory function of HCMV UL141 is associated with its ability to bind diverse proteins, while utilizing at least two distinct binding sites to selectively engage TRAIL DRs or CD155. Binding studies revealed high affinity interaction of UL141 with both TRAIL-R2 and CD155 and low affinity binding to TRAIL-R1. We determined the crystal structure, which revealed that UL141 forms a homodimer that engages two TRAIL-R2 monomers 90° apart to form a heterotetrameric complex. A 'dimerization-deficient' mutant of UL141 (ddUL141) was further designed, which retained the ability to bind to TRAIL-R2 or CD155 while losing the ability to cross-link two receptor monomers. Structural comparison of unliganded UL141 with UL141 bound to TRAIL-R2 further identified a mobile loop that makes intimate contacts with TRAIL-R2 upon receptor engagement. Superposition of the Ig-like domain of UL141 on the CD155 ligand T-cell immunoreceptor with Ig and ITIM domains (TIGIT) revealed that UL141 can potentially engage CD155 similar to TIGIT by using the C'C" and GF loops. Further mutations in the TIGIT binding site of CD155 (Q63R and F128R) abrogated UL141 binding, suggesting that the Ig-like domain of UL141 is a viral mimic of TIGIT, as it targets the same binding

site on CD155 using similar 'lock-and-key' interactions. Sequence alignment of the UL141 gene and its orthologues also showed conservation in this highly hydrophobic (L/A)X6G 'lock' motif for CD155 binding as well as conservation of the TRAIL-R2 binding patches, suggesting that these host–receptor interactions are evolutionary conserved. The breadth of UL141-mediated effects indicates that HCMV has evolved sophisticated strategies to evade the immune system by modulating multiple effector pathways.

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References: Nemčovičová I., et al. 2013-16.

New therapeutic approaches

PHARMACOKINETIC CHARACTERISTICS OF HYPERIMMUNE GLOBULIN TREATMENT IN PREGNANCY: IMPACT ON IGG AVIDITY MATURATION AND NEUTRALIZATION (ID 102)

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Abstract

a) Brief introduction. In contrast to viral reinfection or reactivation during pregnancy, the maternofetal transmission rate at primary infection with CMV is quite high, in the range of 30-50%. About 10-15% of prenatally infected infants acquire long term sequelae like hearing loss. For prevention of maternofetal CMV transmission, a CMV specific hyperimmune globulin (HIG) preparation has been used successfully in several studies (Nigro et al., 2005; Buxmann et al., 2012). In contrast, the first randomized, placebo-controlled clinical trial revealed no statistically significant reduction of CMV using an identical study design (Revello et al., 2014), although, a 14% reduction of the numbers of congenitally CMV-infected newborns was observed. To control the efficacy of HIG therapy longitudinally during primary infection, monitoring of CMV IgG levels, IgG avidity maturation and viral neutralisation are important.

b) Case report. We investigated the influence of HIG administration regimen on the CMV IgG avidity maturation, the IgG levels, the epitope specificity of CMV IgG against recombinant antigen, and on the neutralisation capacity using epithelial cells and a clinical viral isolate from amnion fluid. First, we performed pharmacokinetic analysis of an index case with HIG treatment cycles via monthly doses of 100PEIU/kg/dose of HIG. We found largely fluctuating levels of CMV IgG following HIG administration, with peaks and troughs returning to almost baseline level. We also found repeatedly reductions following HIG administration in CMV IgG response against IE1/p150/CM2 recombinant antigens, and CMV neutralisation titers (NT) and NT50 values against ARPE-19 cells in vitro (Hamprecht et al., 2014).

Based on this observation we changed the administration regimen of HIG treatment to biweekly intervals. Blood sampling for detailed pharmacokinetic analysis were also gained at different time points, and especially at the first treatment cycle at -15min, +30min, +2h, +7d, +14d. Up to now, 36 women were included in the analysis. For all, we found a common time-concentration profile of CMV-specific antibodies confirming our initial observation of a clearly shorter half-life than expected. Fluctuations in individual CMV neutralisation capacity were also observed. Additionally, initial CMV IgG levels and gestational age at beginning of HIG treatment were evaluated.

c) Conclusion. The maternal antibody response to biweekly administrated HIG doses result in a more sustained CMV neutralisation capacity than the usual 4 week-intervals. Additionally, an increase of the trough level of CMV IgG can be observed after multiple dosing when reducing the dosing interval, while for the monthly HIG regimen all CMV antibody parameters under investigation go down to baseline levels. The shortening of the initial HIG treatment schedule might have important implications for the HIG efficacy to prevent maternofetal CMV transmission. Primary endpoint of our clinical study arm is the prevention of maternofetal CMV transmission, documented by amniocentesis. Results will be evaluated still in 2016.

Table / Image

Fig 1a

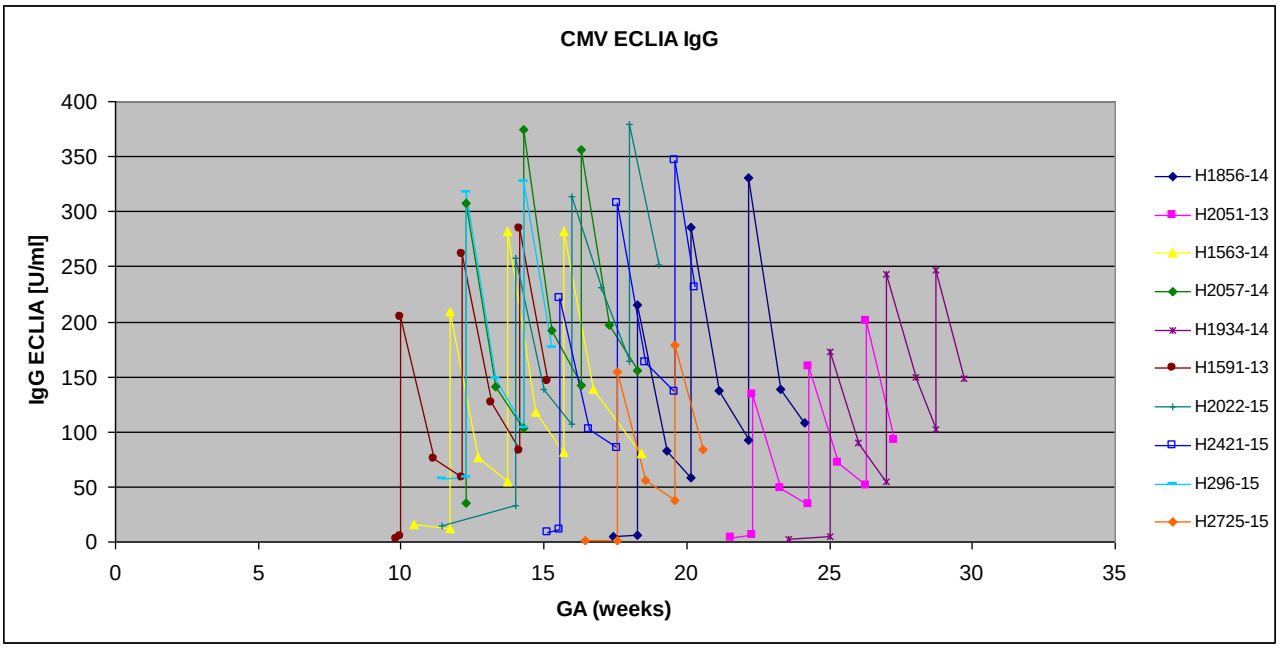
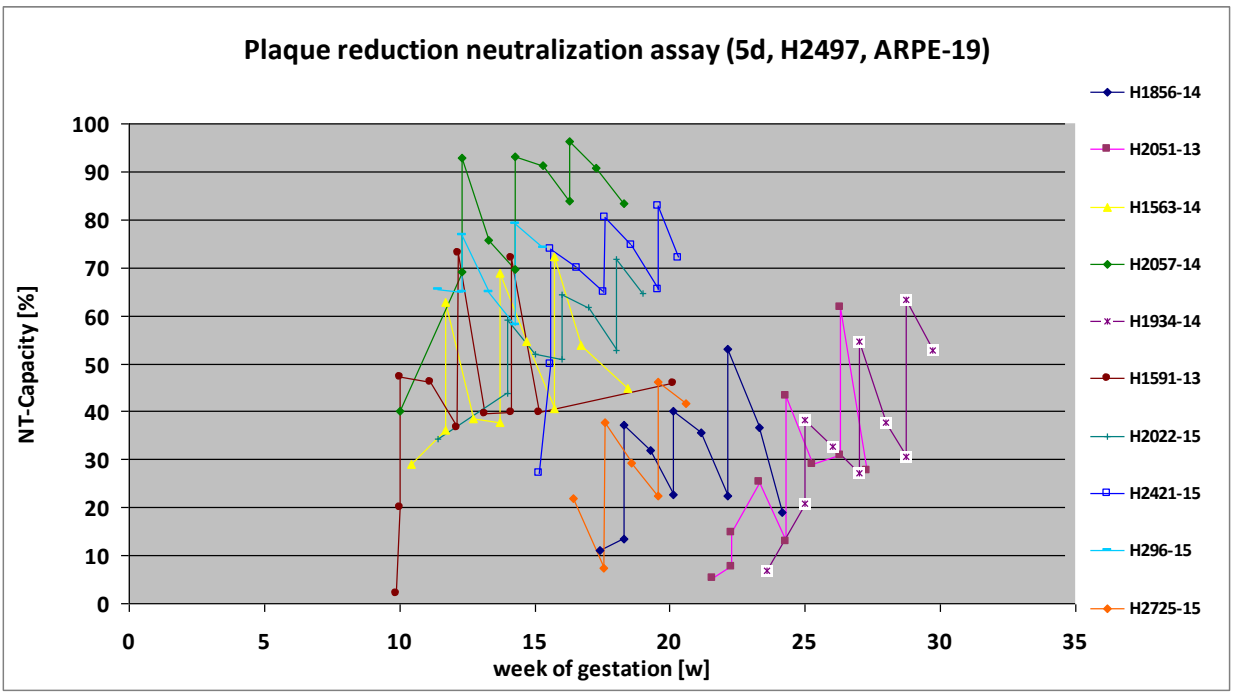


Fig 1b



Monitoring of individual CMV IgG and neutralization capacity during 3 cycles of HIG administration with beweekly interval

Prevention

TEST ACCURACY STUDY OF CURRENT CMV-IGG ASSAYS (ID 075)

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Abstract

Background. Congenital CMV infection is one of the most common causes for permanent disabilities in industrialized countries. Seroprevalence studies show that about 50% of women in childbearing age are susceptible to CMV primary infection. CMV-IgG testing in early pregnancy is recommended by the German guideline for diagnosis of pregnancy associated viral infections. In case of a negative result, hygiene consulting should be offered to prevent infection. To avoid a false sense of security CMV-IgG-assays must be highly specific, however test accuracy studies for current CMV-IgG assays are not available. Materials and Methods. We analysed 1004 consecutive unselected samples, including 112 samples of pregnant women, with the Abbott Architect CMV IgG, DiaSorin LIAISON CMV-IgG II, Medac CMV-IgG-ELISA and Siemens Enzygnost Anti-CMV/IgG. Discrepant samples were tested in a blinded manner by a set of confirmatory assays. Results. Overall 475 samples tested positive and 462 were negative in all four IgG assays. A total of 67 samples had discrepant results of which only three were confirmed positive. False positive measurements were most frequent with Abbott Architect (n=20) and Medac EIA (n=18), followed by DiaSorin Liaison (n=5) and Siemens Enzygnost (n=2). Four, five and 14 samples had borderline results in the Liaison, Enzygnost and Medac assays respectively, all were true negatives. Calculated specificities were between 93.7 and 98.6% (including borderlines) and 96.1 to 99.6% excluding borderline results. False negative results were seen with Diasorin Liaison (2/473) and Medac (1/473). ROC analyses showed that adjustment of the cut-off would result in a better specificity without loosing sensitivity. Conclusion. Performance of some widely used CMV-igG assays could be improved. With the current cut-off definition up to 4 of 100 pregnant women would get a false positive result and therefore remain at risk for CMV infection.

Prevention

EVALUATION OF ANTIVIRAL PRESCRIPTION FOR THE TREATMENT OF CYTOMEGALOVIRUS INFECTION ON NCBMT (ID 086)

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Abstract

Introduction: Viral infections are extremely common and often severe complication of bone marrow transplantation. In particular, Cytomegalovirus (CMV) infection, considered an important cause of morbidity and mortality in recipients of an allograft or autograft. This can partly justify the increased consumption of antiviral most of which are expensive molecules with significant budget share of the institution.

Case report: This is a retrospective study, type review appropriateness of care targeting all systemic antiviral prescriptions to prevent or treat CMV infection, lasting five years from 2009 to 2014, conducted in the hematology and transplant service of the National Bone Marrow Transplant Center. The results showed that 68% of prescriptions were deemed fully compliant with the commendations for the primary endpoint (indication) and all secondary endpoints (administration modalities). Non-compliance reside in the criterion of the choice of the molecule, dosage and the route of administration with a rate of 2.2%. While a rate of 3.7% was reported for the criterion of the duration of treatment, and 4.5% of prescriptions were deemed non-compliant for the criterion of the association to antivirals. Based on our analysis it was shown that non-conformities reside in the methods of administration of antivirals, mainly in the choice of the molecule that is a very important criteria from a clinical and pharmaco-economic point of view. On the other hand, it was found that the internal protocol and the international recommendations have some differences that need to be studied.

Conclusion: The non-conformities identified during this work allowed to define the priorities that will be the starting point of a quality approach to improve the formulation of prescriptions.

Prevention

KNOWLEDGE OF HCMV INFECTION IN PREGNANT WOMEN AT A TERTIARY CARE CENTER DEMONSTRATES THE NEED OF INFORMATION AND PREVENTATIVE CAMPAIGNS. (ID 100)

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Abstract

Introduction

Primary Human Cytomegalovirus (HCMV) infection in pregnancy is a leading cause of congenital abnormalities. A recent study (Revello MG et al. EBioMedicine. 2015) provided evidence that primary prevention based on counselling of HCMV seronegative pregnant women significantly prevents maternal infections. There is lack of data on knowledge of pregnant women that support the need and possible utility of such intervention in a real life population.

Results

A questionnaire was administered to all pregnant women attending our institution from November 2015 to February 2016 . Demographical data, level of education and responses to the questionnaire were recorded. Eight multiple choice questions, in particular, assessed knowledge of strategies for preventing HCMV vertical transmission were administered (i.e., avoid cats; avoid raw meat; wash accurately fruits and vegetables; clear carefully hands after garden works; avoid kissing children on mouth; wash hands after touching children's mouth or nose; wash hands after exposure to young children's body fluids as urine; not share food, drinks, washcloths etc.). 183 pregnant women were interviewed. Mean maternal age was 30 years and mean gestational age was 22 weeks. All patients but 6 were Italians. 59.3% women had a secondary school degree, while 26.3% had a University degree. For 51% women, serological tests for HCMV were available (75% of them had positive IgG/negative IgM, 21.9% negative IgG and IgM, and 2% positive IgG and IgM). Among pregnant women 58% knew the existence of HCMV. 81% considered HCMV infections to be dangerous during pregnancy even though many of them were unaware of the virus at a previous question, clearly indicating the inconsistency of information. Moreover, 10% did not considered this infection to be a

potential risk for the fetus and 7% simply did not know. Lastly, at multiple choice question, only 34% of the patients gave at least 4 right answers about the means to avoid HCMV infection.

Conclusion

Our study clearly demonstrates the lack of knowledge of HCMV in pregnancy and the urgency to implement preventative campaigns targeted both to women and doctors. Even though Italian guidelines does not recommend HCMV serology before and during pregnancy, most women received it but these tests simply appears to be a missing opportunity in at least 1:5 seronegative women who had required an appropriate counselling intervention.

Prevention

ACCEPTANCE OF AND ADHERENCE TO HYGIENE MEASURES TO PREVENT PRIMARY CYTOMEGALOVIRUS INFECTION IN PREGNANCY (ID 115)

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Abstract

Background. Cytomegalovirus (CMV) is the most frequent cause of congenital infection. Primary infection in pregnancy carries the highest risk of congenital disease including mental retardation, motor disabilities and hearing loss. No vaccine is currently available and treatment options during pregnancy are limited. Recently, we showed in a controlled trial that an intervention based on the identification and hygiene counseling of CMV-seronegative pregnant women significantly reduced the rate of seroconversion in pregnancy (EBioMedicine 2015;2:1205-10). Acceptance of and adherence to the educational intervention by the women who participated in the trial were investigated.

Methods. Seronegative women at high risk for primary CMV infection due to frequent contact with children of <36 months of age for personal or professional reasons, were identified at 11-12 weeks' gestation (WG). Women received detailed information about the virus, routes of transmission, potential consequences to the fetus and preventive measures. Specifically, women were invited to adopt protective measures (frequent hand washing) and to avoid risky behaviours (kissing children on the mouth/cheeks, sharing utensils, food, drinks, washcloths). To assess acceptance of and adherence to suggested hygiene measures a questionnaire was developed. Women were invited to fill in the questionnaire every 6 weeks starting at 18 WG and, in the last questionnaire at 37 WG, to provide and justify a final statement about the intervention received.

Results. Of the 331 seronegative pregnant women who received CMV counseling at 11-12 WG and completed the study, 255 (77%) completed at least one questionnaire and 219 (66%) completed the questionnaire at 18 and 37 WG. Overall, 932 completed questionnaires were returned. Family was composed of 3 members in about 70% cases. Partners were reported to help most of the time/always with housework and care of child(ren) by 65% women. About 65% women reported receiving additional help mainly by grandparents (70% of cases). Eighty-four percent women shared the

hygiene information received with the closest person(s) and 73% women found this useful in following recommendations. About 80% of respondents reported following the recommendations most of the time (66%) or always (14%) during pregnancy. Major difficulties were lack of time (43% respondents), and child(ren) reactions (about 30% respondents). Knowledge of the risks to the fetus helped the most in following recommendations (74% respondents at 18 WG and 64% at 37 WG). Notably, regular adoption of hygiene measures increased during pregnancy from 27% to 44% of respondents. Finally, 93% of respondents considered the educational intervention received worth suggesting to all pregnant women at risk for CMV infection.

Conclusions. Substantial or complete compliance with suggested recommendations was reported by the majority of women. CMV counseling of seronegative women at risk of infection appears an acceptable and highly effective strategy for primary prevention of congenital CMV.

Primary and non-primary infection in the mother

PRESENCE OF CYTOMEGALOVIRUS IN URINE AND BLOOD OF PREGNANT WOMEN MIGHT BE ASSOCIATED WITH INFECTION ON OFFSPRING (ID 079)

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Abstract

Background: Cytomegalovirus (CMV) congenital infection can result from primary infection, reinfection or reactivation among pregnant women. The risk of vertical transmission is known to be higher in case of primary infection, and the transmission rate increases with gestational age.

However there are still many questions about maternal markers that can predict whether the virus will be transmitted to the offspring.

Objectives: To investigate the relationship between the presence and the quantity of CMV in urine and blood of women presenting a primary CMV infection during pregnancy and the presence of congenital infection in their offspring.

Study design: Detection and quantification of CMV DNA was performed on 150 urine samples and 114 blood samples from 150 pregnant women with proven CMV primary infection. The relationship between the presence of CMV in maternal blood and urine and infection in offspring was calculated with OR and 95%CI. The relationship between the quantity of virus in blood and urine and infection in offspring was calculated using a Wilcoxon Manning test.

Results: Transmission rate was 36,7%. A marginally statistically significant association was found between the presence of CMV in maternal urine and infection in offspring (OR 2.03 95%CI 1.03-3.99). A clearly significant association was found between the presence of CMV in maternal blood and infection in offspring (OR 3.14 95%CI 1.38-7.16). Taking into consideration those samples that are positive for CMV in maternal urine, the median value of viral load was significantly higher in those patients who transmitted to offspring (p50 4480, p25 2060- p75 11100) compared to those who didn't (p50 2570, p25 598- p75 4345).

No significant association between viral load in maternal blood and infection in offspring was observed.

Conclusion: the presence of CMV in maternal urine and maternal blood correlates in our sample to the transmission of CMV to offspring. The viral load found in urine of mothers who transmitted CMV to their newborn is statistically higher than the viral load in urine of mothers who didn't transmit.

Basic science developments and opportunities for translational research

INNATE NUCLEAR SENSOR IFI16 TRANSLOCATES INTO THE CYTOPLASM DURING EARLY STAGE OF IN VITRO HCMV INFECTION AND IS ENTRAPPED IN THE EGRESSING VIRIONS DURING LATE STAGE (ID 018)

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Abstract

Intrinsic immune mechanisms constitute frontline antiviral defense mediated by constitutively expressed proteins termed "restriction factors". We recently demonstrated that the DNA sensor IFI16 restricts HCMV replication by down-regulating viral early and late but not immediate-early mRNAs and their protein expression (Gariano GR, Dell'Oste V et al Plos Path 2012). Here, we show that at an early time point during the in vitro infection of low-passage human embryonic lung fibroblasts (HELF) IFI16 binds to HCMV DNA. However, at a later phase following infection, IFI16 is mislocalized to the cytoplasmic assembly complex (AC), where it colocalizes with viral structural proteins. Upon pUL97 binding, indeed, IFI16 undergoes phosphorylation and relocalizes into the cytoplasm of HCMV-infected cells. ESCRT (Endosomal Sorting Complex Required for Transport) machinery regulates the translocation of IFI16 into the virus AC by sorting and trafficking IFI16 into multivesicular bodies (MVB), as demonstrated by the interaction of IFI16 with two MVB markers: Vps4 and TGN46 (Dell'Oste et al. JVI 2014). Finally, IFI16 becomes incorporated into the newly assembled virions as demonstrated by Western blot analysis and electron microscopy of purified virions, obtained from both laboratory strains and clinical isolates (kidney transplant recipients and children with congenital CMV infection). Together, these results suggest that HCMV has evolved mechanisms to mislocalize and hijack IFI16 into mature virions. However, the significance of this IFI16 trapping following nuclear mislocalization remains to be established.

Basic science developments and opportunities for translational research

PERINATAL MOUSE CYTOMEGALOVIRUS INFECTION INDUCES ACTIVATION OF BRAIN RESIDENT MICROGLIAL CELLS AND RECRUITMENT OF INFLAMMATORY CELLS INTO THE BRAIN (ID 057)

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Abstract

Introduction:

Congenital human cytomegalovirus (HCMV) infection is the most common viral cause of long-term neurodevelopmental sequelae, including mental retardation, microcephaly and sensorineural hearing loss. As HCMV does not cross the species barrier, we employed a mouse model in which newborn mice are infected by intraperitoneal (i.p.) inoculation of mouse cytomegalovirus (MCMV). Following infection the virus disseminates to the central nervous system (CNS) and replicates in the brain parenchyma. CNS infection leads to the activation of resident microglial cells and the recruitment of peripheral immune cells. In addition, the virus induces delay in cerebellar growth.

Results:

In our model of congenital MCMV infection, the initial neuroimmune responses are dominated by activation of resident microglial cells, characterized by upregulation of MHC molecules, iNOS production and the influx of NK cells, whose appearance coincides with detection of the virus in the brain. The number of NK cells in the CNS peaked at day 8 post infection (p.i.). Phenotypic analysis showed that brain infiltrating NK cells are highly activated and produce IFNγ. In addition, we also observed recruitment of other peripheral immune cells, of which CD8+ T cells were the most numerous and peaked on day 21 p.i. Phenotypic analysis showed that MCMV-specific CD8+ T cells are highly activated and display tissue resident memory phenotype during latency. Furthermore, after adoptive transfer of CD8+ T cells isolated from MHC class I restricted TCR transgenic mice which are specific for the MCMV-derived epitope m38, we demonstrated that upon MCMV infection, CD8+

T cells migrate from the periphery to the brain. Importantly, adoptively transferred transgenic cells counteract the virus-induced delay in granular neuron migration in the cerebellum of MCMV-infected newborn mice.

Conclusions:

Our results demonstrated the coordinated action of innate and acquired immunity in the clearance of congenital MCMV infection of the brain. Furthermore, the results demonstrated the functional role of antigen-specific CD8+ T cells in protection from virus-induced pathology in the neonatal CNS.

Basic science developments and opportunities for translational research

NOVEL CLUES IN ANTI-CYTOMEGALOVIRUS DRUG TARGETING BASED ON PHOSPHORYLATION-TRIGGERED NUCLEAR HOST CELL EGRESS (ID 013)

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Abstract

Nuclear replication of human cytomegalovirus (HCMV) and other herpesviruses relies on elaborate mechanisms of nuclear virus-host interaction. Many nuclear processes in HCMV replication are dependent on site-specific protein phosphorylation, as exerted by viral pUL97 and cellular protein kinases. This applies in particular to the nucleocytoplasmic export of viral particles, for which the role of two essential and conserved viral nuclear proteins, pUL50 and pUL53, is pivotal. We and others demonstrated that pUL50 and pUL53 heterodimerize and form the core nuclear egress complex (NEC), which is anchored to the inner nuclear membrane and provides a scaffold for the assembly of a multimeric viral-cellular NEC. This is considered as a highly conserved transport and recruitment process, since pUL50-pUL53 assembly at the nuclear envelope could even be detected in a heterologous host system (plant cells). Importantly, a first crystal structure of the pUL50-pUL53 core NEC was resolved at 2.44 Å resolution, revealing several unique and functionally relevant features. Proteomic analyses emphasized the importance of NEC-associated proteins, including the viral protein kinase and a number of cellular and viral regulatory factors. Data from functional analyses, including the use of recombinant HCMV carrying NEC mutations and siRNA approaches, led to a successful validation of NEC proteins for preventive strategies. Based on our findings, novel

mechanistic clues were obtained to utilize this viral-cellular protein complex as a target for antiviral drug design, and to exploit the plurality of NEC functions as attack points for small molecule-type inhibitors. First experiments based on a small screening of prototype inhibitors demonstrated that the nuclear rim recruitment of pUL50-pUL53 can be antagonized by the treatment with protein kinase inhibitors. We are currently working on further concepts (i) for interfering with protein kinase activity (pUL97 and cellular), (ii) for steric hindrance of hook-to-groove interaction or multi-ligand binding, respectively, and (iii) for antagonizing conformational change and distortion of the nuclear lamina. Thus, this study may accelerate the validation of herpesviral NEC structures as a unique target for developing a novel type of antiviral drugs.

Basic science developments and opportunities for translational research

CHARACTERIZATION OF HUMAN CYTOMEGALVIRUS IE1 MUTANTS REVEALS THAT VIRAL TARGETING OF PML BODIES PERTURBS BOTH INSTRINSIC AND INNATE IMMUNE RESPONSES (ID 026)

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Abstract

PML is the organizer of cellular structures termed nuclear domain 10 (ND10) or PML-nuclear bodies (PML-NBs) that act as key mediators of intrinsic immunity against human cytomegalovirus (HCMV) and other viruses. During HCMV infection, the major immediate early protein IE1 binds to PML via a central globular domain (IE1CORE) as revealed by the recent solution of the crystal structure of primate IE1 proteins and we have shown previously that this is sufficient to antagonize intrinsic immunity. Here, we demonstrate that modification of PML by IE1CORE not only abrogates intrinsic defense mechanisms, but it also attenuates the interferon response during infection. Our data show that PML plays a novel co-regulatory role in type-I as well as type-II interferon-induced gene expression which is antagonized by IE1CORE. Importantly, our finding supports the view that targeting of PML-NBs by viral regulatory proteins has evolved as a strategy to inhibit both intrinsic and innate immune defense mechanisms. Consequently, interference with PML-binding of IE1 may constitute a novel antiviral strategy to inhibit HCMV replication at a very early step.

Basic science developments and opportunities for translational research

EARLY NEUROIMMUNE EVENTS INCLUDING MICROGLIAL ACTIVATION FOLLOWING IN UTERO CMV INFECTION IN THE DEVELOPING RAT BRAIN (ID 042)

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Abstract

Brief introduction

Congenital CMV infections are a major cause of neurodevelopmental disorders in human and represent an important health care and socio-economical burden. In contrast with this medical importance, the pathophysiological events associated with brain CMV infection remain poorly known. Murine models of brain CMV infection, notably neonatal, have brought recent insights into the possible pathogenesis, with convergent evidence for the alteration and for the possible involvement of brain immune cells. Studies in rodent models which would take into account in utero events, are still needed to confirm and expand those findings, particularly concerning the early developmental stages and neuroimmune events following infection of the fetal brain, and the postnatal neurological outcome.

Results

We have created a novel model of in utero CMV infection in the developing rat brain. GFP recombinant rat CMV was injected intracerebroventricularly at embryonic day 15 and the brains analyzed using a combination of gene expression analysis, immunohistochemistry and multicolor flow cytometry experiments. CMV was detected in an increasing number of brain areas

(periventricular areas, choroid plexi, olfactory bulbs, inner ears...) and cells from E17 to P1. CMV was prominently detected in CD45low/int CD11b+ immune cells corresponding to microglial cells, in CD45high CD11b+ cells (myeloid lineage cells including macrophages), and in CD45high CD11b– cells (lymphocytes, NK and non-B non-T cells). Rat CMV infection of the developing rat brain rapidly triggered a cascade of events comprising chemokine dysexpression and infiltration by peripheral immune cells; also, early infection and activation of microglial cells - the resident mononuclear phagocytes of the brain - were observed.

Conclusion

Neuroimmune events and notably microglial infection and activation occurred early upon CMV infection of the developing brain in utero. In line with previous findings in neonatal murine models and in human specimen, our study further suggests that the aforementioned pathophysiological events might play critical roles in the early stages following brain CMV infection in utero. Based on ongoing pharmacological interventions and postnatal neurological evaluations, we have already obtained promising data in order to determine which role, whether favorable or detrimental, those microglial events might actually play in the pathogenesis of congenital CMV infection of the brain and in the emergence of neurodevelopmental phenotypes.

CMV in Europe

THE ISRAELI EXPERIENCE WITH CONGENITAL CMV IN INFANTS BORN TO MOTEHRS WITH PRIMARY INFECTION COMPARED TO PUBLISHED DATA FROM THE USA. (ID 004)

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Abstract

Nearly 10-15% of infants with congenital CMV (cCMV) infection born to mothers with primary infection will be symptomatic at birth and about 20-25% will developed sequelae.

In Israel about 2/3 of pregnant women are screened for CMV and those with primary infection are followed at high risk clinics. Part of these pregnancies are terminated because of abnormal fetal imaging or maternal request. The indications for antiviral therapy in Israel are more extensive than used in most centers treating cCMV. The aim of this study is to compare the outcome of these infants in Israel to the data from the USA.

Methods: A retrospective multicenter study of Infants with cCMV that included 60-80% of this population in Israel borne on 1997-2013. Inclusion criteria: cCMV, primary maternal infection and follow-up >1 year. Historical control: published data from USA.

Results: 419 infants were included; 131 infants were born asymptomatic and 288 symptomatic. The main post-natal finding in comparison to data from the USA are presented in table 1.

Table 1.

Clinical sign	Israel	USA	P
IUGR	48/148* (32%)	53/106 (50%)	0.004
Petechia	11/148 (7%)	80/106 (76%)	<0.001
Microcephaly	39/148 (26%)	54/102 (55%)	<0.001
Thrombocytopenia	33/148 (22%)	62/81 (77%)	<0.001
Hepatitis	14/145 (10%)	46/58 (83%)	<0.001

*the number of cases are according to the definition of symptomatic cCMV in each study.

The data on neurological sequelae are presented in table 2.

Clinical sign	Israel	USA	P
Hearing loss	27/125 (22%)	58/100 (58%)	<0.001
Chorioretinit	6/145 (4%)	19/93 (20%)	<0.001
Mental Retardation	15/136 (11%)	33/60 (55%)	0.029
Seizures	7/136 (5%)	24/104 (23%)	<0.001
Long term sequelae	46/121 (38%)	70/122 (57%)	0.002

Conclusions: the outcome of infants with cCMV infection after primary maternal infection is significantly better in Israel than the published data from the USA. More studies are needed to understand the factors responsible for this results.

CMV in Europe

eVLP DELIVERY OF AN OPTIMIZED FORM OF CMV gB ANTIGEN FOR PROPHYLACTIC VACCINATION AGAINST CONGENITAL CMV (ID 049)

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Abstract

Introduction

A prophylactic vaccine to prevent congenital transmission of human cytomegalovirus (HCMV) to newborns and to reduce life-threatening disease in immunosuppressed recipients of HCMV-infected solid organ transplants would be highly desirable. Neutralizing antibodies against HCMV confer significant protection, and gB is a major target of such neutralizing antibodies. However, one shortcoming of past HCMV vaccines may have been a failure to induce high titer persistent neutralizing antibody responses that could prevent infection of epithelial cells.

Results

We have used enveloped virus-like-particles (eVLPs), in which particles were produced in HEK 293 cells after transient expression of murine leukemia virus (MLV) viral matrix protein Gag, to express the full extracellular domain of CMV gB fused with the transmembrane and cytoplasmic domains from vesicular stomatitis virus (VSV) G protein (gB-G eVLPs). Cryo-EM analysis of the surface of eVLPs expressing native gB vs. the gB-G form of antigen demonstrate different structures, which is further confirmed by differential reactivity to antibodies generated against gB and gB-G forms of the antigen. eVLP expression of the gB-G form of antigen is associated with a 5-fold improvement in neutralizing titers relative to native gB. Immunization of two animal species (mice and rabbits) demonstrates that after two doses (weeks 0 and 8) gB-G eVLPs adsorbed to alum induce neutralizing antibody titers against fibroblast and epithelial cell infection that exceed or are equivalent, respectively, to naturally acquired levels of immunity. In vivo testing of multiple (n=3) 10L batches produced during the tech transfer process to our CMO has demonstrated consistent potency, and analytical testing of toxicology and clinical batches at 50L scale further confirms manufacturing consistency. Stability studies demonstrate drug product stability for 18 months at 4C. The vaccine candidate has been purified to meet criteria established by the FDA for licensed human vaccines.

Conclusions

eVLP expression of an optimized form of gB antigen subsequently absorbed to alum represents a novel approach to developing a potentially efficacious prophylactic CMV vaccine. Clinical evaluation of this candidate in a phase I study is planned to begin in H1 2016.

CMV in Europe

CONGENITAL CYTOMEGALORIVUS INFECTION AND COMORBID CONDITIONS (D 055)

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Abstract

INTRODUCTION:

Although cytomegalovirus (CMV) is the most common congenital infection, existing research has not provided us with a full picture of how this can affect children in the future. The aim of this case-control study was to evaluate disabilities in a well-defined group of children with congenital cytomegalovirus (CMV) infection, who had been fitted with cochlear implants because of severe hearing impairment.

METHODS:

A multidisciplinary team assessed 26 children with congenital CMV infection for balance difficulties, neurodevelopmental disabilities and language and visual impairment. We also included a control group of 13 children with severe hearing impairment due to connexin 26 mutations.

RESULTS:

The majority of the children with congenital CMV infection (88%) displayed balance disturbances, including walking at a later age, but there were no cases in the control group. The CMV group also displayed frequent neurodevelopmental disabilities and feeding difficulties.

CONCLUSION:

Congenital CMV infection affects the general development of the brain and gives rise to a complex pattern of difficulties. Identifying comorbid conditions is very important, as children with associated difficulties and disabilities need more support than children with just hearing impairment. Congenital CMV infection needs to be considered in children with hearing impairment and/or balance disturbance and/or neurodevelopmental disabilities.

CMV in Europe

EXECUTIVE FUNCTIONS IN CHILDREN WITH COCHLEAR IMPLANTS AND CONGENITAL CYTOMEGALOVIRUS INFECTION – PRELIMINARY FINDINGS (ID 050)

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Abstract

Congenital cytomegalovirus (cCMV) infection is one of the most common congenital infections that may result in a progressive hearing loss (HL) or deafness. In a recent study of Swedish children with hearing impairment (HI) caused by cCMV infection, around 90 % had severely damaged balance function leading to e.g. late on-set of walking (Karltorp et al., 2014). Around 20 % had vision impairment, 15 % were diagnosed with Autism-Spectrum-Disorder (ASD) and 20 % with ADHD. One interesting clinical observation during the initial study was that the majority of children with cCMV infection displayed problems with their executive functions (EF) like impaired impulse control and poor attention while controls with connexin (cx) 26 caused HI did not display similar difficulties. Therefore, a new prospective study was initiated with the main purpose of examining EF with more formal assessment tools and with a interdisciplinary team approach. Twenty-one children with cCMV infection aged 1:10-18:3 years and eleven children with cx26 aged 1:0-14:7 years participated in the study. The majority of participating children had bilateral CIs. Results showed that children with HI caused by cCMV infection had more difficulties with EF skills than HI controls (cx26). However, there was a large variation within the group of children with cCMV infection. Preliminary results from the current study will be presented and in addition some clinical implications of the overall outcome will be discussed during the presentation.

CMV in Europe

BREASTMILK CYTOMEGALOVIRUS TRANSMISSION IN VERY LOW BIRTH WEIGHT INFANTS AND USEFULNESS POLYMERASE-CHAIN REACTION (PCR) ASSAY WITH GUTHRIE CARD SAMPLES TO DIFFERENTIATE CONGENITAL AND POSTNATAL INFECTIONS: CASE REPORTS. (ID 048)

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Abstract

Cytomegalovirus (CMV) infection is the most common congenital infection and may lead to death or neurologic sequelae and sensorineuronal hearing loss. CMV may also be acquired postnatally by blood transfusion or infected breastmilk. Being born at ≤28 weeks gestational age or with a birth weigh <1500 g are most important risk factors for infants CMV for disease caused by postnatally acquired infection presumably because of deficiency in passive transfer of maternal antibodies. Although postnatal CMV infections has no consequences in term newborns, it can cause symptomatic disease in immunologically immature, very low birth weight (VLBW) infants (birthweight ≤1500 grams). Symptomatic postnatal CMV infection is characterized by hepatopathy, thrombocytopenia, neutropenia, petechia, respiratory distress syndrome, and sepsis-like syndrome .

We report three WLBW preterm and three ELBW infants fed with breast milk with postnatally acquired CMV disease. To prove postnatal transmission of cytomegalovirus infection, CMV DNA PCR test was applied with Guthrie card blood samples received a week after birth.

CMV PCR of dried blood spots on Guthrie card which was taken within 1 week after birth is useful methods to exclude congenital infection and to proof for postnatal transmission.

CMV in Europe

PROSPECTIVE cCMV SCREENING OF NEWBORNS IN ATHENS. PREVALENCE AND OUTCOME. (ID 039)

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Abstract

Congenital cytomegalovirus infection (cCMV) represents the most common congenital infection. Incidence of congenital CMV infection varies between 0.2% and 2.5% of all live births but most newborns are asymptomatic. Although both intrauterine transmission and sequelae (mainly SNHL) are more common among women with primary CMV infection during pregnancy it has been well documented that there is a considerable risk among infants born to seropostive pregnant women with reccurent infection. Universal neonatal screening, although expensive would enable early diagnosis and intervention but would prohibit any therapeutic intervention during pregnancy.

We developed a modified DNA extraction method for the quantification of CMV-DNA in Guthrie cards and prospectively screened newborns in two major maternity hospitals in Athens Greece (2008-2010). Demographic and maternal CMV serologic data was collected. Overall, 2149 newborns were enrolled. Median maternal age was 32 years, 78% of mothers were of Greek origin, 73% CMV seropositive during prenatal screening, while only one woman seroconverted during her third trimester. Median birth age and weight were 38+2 weeks and 3.270 gr respectively. Prevalence of CMV-DNA in Guthrie card was 0.51% (11/2149). cCMV babies were examined and prospectively followed for five years. All babies were asymptomatic at birth with normal auditory brainstem response and cranial US. Most (10/11) were born to women with documented CMV-seropositivity during prenatal screening. The seronegative mother had not been re-evaluated during pregnancy. None received antiviral treatment. At five years of age, two had significant bilateral SHL (one had cochlear inplant and the other used hearing aid in both ears). All five had normal neurologic examination and psychomotor development.

In conclusion, similarly to other European countries most children with cCMV are born ot seropositive women. Although asymptomatic, almost 20% had sensorineural hearing loss.

CMV in Europe

SUBVIRAL DENSE BODIES OF THE HUMAN CYTOMEGALOVIRUS CONTAINING THE GH/GL/UL128-131A COMPLEX AS THE BASIS FOR VACCINE DEVELOPMENT (ID 023)

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Abstract

Development of preventive strategies against congenital human cytomegalovirus (HCMV) infection is a challenging task. Several vaccine candidates are currently investigated with respect to their efficacy to prevent primary and recurrent HCMV infection during pregnancy. We focused our attention on the use of subviral Dense Bodies (DBs) of HCMV for that purpose. It is commonly accepted that an effective HCMV vaccine should target both cellular and humoral antiviral responses. We could recently show DBs stimulate the maturation and activation of monocyte-derived immature dendritic cells (Sauer et al., JVI 2013). This explained the exceptional T-cell responses following DB-application. In this study, we addressed the humoral response to DBs with a particular focus on the role of neutralizing antibodies (NT-abs) directed against the viral envelope complex gH/gL/UL128-131A (pentamer). This complex determines the wide host cell range of HCMV infection. It is expressed on virions of clinical isolates, but not on virions of laboratory strains of HCMV, thus disabling infection of endothelial or epithelial cells by the latter viruses. However, mass spectrometry revealed that even pentamer-positive strains like TB40/E express only limited amounts of that complex on virions (Büscher et al., MMI 2015). We thus first addressed if pentamer-negative DBs of the laboratory strain Ad169 induced an NT-abs response against infection of epithelial and endothelial cells. Surprisingly, these sera lacking pentamer-specific antibodies neutralized TB40/E on these cells to some extent. In a second step, a pentamer-positive variant of HCMV laboratory strain Towne was generated by BAC-mutagenesis (Towne-rep). DBs released from Towne-rep-infected HFF contained the pentamer. Immunization of mice with these particles induced antibody responses with considerable neutralization capacity on epithelial and endothelial cells. Data comparing the NT-abs response directed against DBs-Towne and DB-Towne-rep will be presented.

CMV in Europe

AWARENESS ABOUT CMV AND CONGENITAL CMV INFECTION IN A ITALIAN POPULATION (ID 031)

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Abstract

INTRODUCTION: Cytomegalovirus (CMV) is a very important a cause of congenital infection (cCMV). Despite its dangerousness and widespread, awareness about the infection and the virus seems to be limited.

METHODS: We designed and developed a survey, conducted by a Computer-assisted web interviewing (CAWI), inviting 70975 people, aged 18-70, to complete the questionnaire. They were interviewed in order to deduce the knowledge about CMV and cCMV infection and its correlation with socio-demographic variables in an open population.

RESULTS: Out of the 10,190 respondents, 5,351 (52.5%) had already heard about CMV but only 3,216 (31.8%) knew that CMV could be implicated in congenital infection. Urine and breastfeeding were the lowest recognized transmission routes of CMV infection; less than half of interviewees accurately identified the right symptoms and sequelae due to cCMV infection. The correct hygienic measures against cCMV infection were identified in a range from 47% to 64% depending on the items proposed but about 1/3 of respondents deemed them unnecessary if a pregnant woman was already CMV seropositive. No significant difference in awareness about cCMV in parents or in parents-to be and in non-parents was observed; high levels of education and childbearing age significantly increased the consciousness of the different topics explored.

DISCUSSION: Our results confirm the limited knowledge about cCMV infection in an open Italian population. To date, educational counselling seems to be the most effective tool in reducing exposure to the virus during pregnancy. Increasing awareness in people about cCMV, is expected both to improve the attention towards CMV infection and to enhance the prevention of its congenital transmission.

CMV in Europe

T-CELLS AND B-CELLS IN NEONATAL DRIED BLOOD SPOTS IN CHILDREN WITH CONGENITAL CMV INFECTION: FINDING PROGNOSTIC MARKERS (ID 011)

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Abstract

Introduction

Among the children with congenital CMV infection (cCMV) with symptoms at birth, around 50% develop long-term sequelae (LTS). Of the asymptomatic group (85%) around 13.5% has consequences. Prognostic markers for clinical outcome help in defining subgroups of patients that would benefit from clinical, audiological follow-up and possibly antiviral treatment. In this study we evaluated whether screening for molecular T- and B-cell markers in Dried Blood Spots (DBS) of children with and without cCMV could serve as prognostic markers.

Materials and methods

We used 99 CMV+ neonatal DBS that were previously tested positive for CMV with a control group of 54 CMV- DBS. From all children, data on LTS at 5 years of age are available (CROCUS study). We set up two-internally-controlled multiplex-real-time PCRs for quantification of T-cell receptor excision circles (TRECs), Vδ1-Jδ1 and Vδ2-Jδ1 TCR rearrangements, intronRSS-Kde coding joint and signal joint KREC relative to β-globin. These measurements reflect the amounts of naïve αβ T cells, mature γδ T-cells, mature and naïve B cells, respectively. A linear mixed model was used in order to assess the difference between the groups taking into account the repeated measurements within a patient. The effect of CMV viral load on these markers was assessed by comparing the high with low viral load group, defined by the first and third quartile, as well as by Pearson's correlation. Furthermore, the group of children that developed LTS (n=24) was compared to the group without LTS (n=75).

Results

TREC copies/µl whole blood were significantly lower in the CMV+ group than the CMV- group ($p=0.043$), and the percentage of alleles that contain the TREC rearrangement normalizing for β-globin was also slightly lower ($p=0.073$). In addition, the CMV+ group had a significantly higher percentage of alleles with the Vδ1-Jδ1 rearrangement (0.019), the intronRSS-Kde cj ($p=0.055$) and slightly higher sj KREC. The high viral load group had a significantly higher percentage of Vδ1-Jδ1 rearrangements ($p=0.022$), intronRSS-Kde cj ($p=0.024$), sj KREC ($p=0.002$) and sj KREC copies/µl whole blood ($p<<0.05$). The Pearson's r analysis indicates a moderate positive correlation, between Vδ1-Jδ1, intronRSS-Kde cj, sj KREC, sj KREC copies/µl whole blood and viral load: $r=0.3$, $r=0.35$, $r=0.38$, $r=4$ and $p<<0.05$, suggesting that higher viral loads lead to higher cellular response. Within CMV+, the group without LTS had a significantly higher % of alleles that contains the sj KREC rearrangement ($p=0.009$) and sj KREC copies/µl whole blood ($p=0.038$) than the group with LTS.

Conclusions

cCMV affects the amount of naïve αβ T- (lower), mature γδ T- and B-cells (higher) measured on DBS. Having higher viral loads seems to increase the number of Vδ1-Jδ1, naïve and mature B-cells. The amount of naïve B cells was significantly higher in the CMV+ that do not develop LTS. sj KREC (% or copies) has been shown to be infection and LTS related. This biomarker appears to be a worthwhile candidate to be further explored with fresh material in a bigger cohort for use as a prognostic marker for clinical outcome.

CMV in Europe

ADAPTIVE AND INNATE IMMUNE RESPONSES IN FETAL HCMV INFECTED BRAIN (ID 028)

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Abstract

Background: Among CMV infected fetuses 10 % to 20% will develop cerebral lesions that can be seen at prenatal ultrasound. In these cases, since the prognosis of severe brain lesions is poor, termination of pregnancy (TOP) is often requested. The physiopathology of CMV induced brain lesions is not completely understood particularly the respective parts of viral multiplication and of immune response are not well characterized.

The aim of this study was to evaluate the adaptive and innate immune responses to the viral multiplication in brains and in placentas obtained from infected fetuses after TOP in relation to the severity of brain lesions.

Materials and methods: Fetal brains and placenta obtained from 21 infected fetuses aged from 23 to 28 weeks and presenting cerebral abnormalities. Brains were classified in A (severe lesions) and B (moderate lesions) and placenta in villitis 1-2 (moderate) and 3 (severe). Five fetuses without cerebral abnormalities and any infection were selected as controls. Immunohistochemistry

(Cd3/cd8/cd20/plasma cells/NK/CMV) was realized in brain and placenta samples. All slides were scanned and the density of immuno-staining was quantified by quantitative image analysis, software developer XD, Definiens Company. Principal composant analyses were realized by R software and Kruskall-Wallis and Fischer tests by Prisme Graph Pad 6.

Results: Principal component analyses of viral and immunologic markers showed a clear repartition between severe, moderate affected brains and normal brains (controls). Viral multiplication, but not immune response, was significantly higher in placenta from fetuses with severe brain lesions than in those with moderate brain lesions. However, there was no significant link between the immune response and the viral multiplication in placenta and the stage of villitis. The immune response and the viral multiplication were both significantly stronger (except for Cd20) in brains with severe lesions than in brains with moderate lesions . In all cases, CMV infected cells as well as NK p46 cells and plasma cells were mainly found in the periventricular zones when CD3 and CD8 staining were spread in the whole cerebral tissues.

Conclusion: CMV multiplication but not immune-response in placenta reflected cerebral severity. There was a clear link between viral multiplication, innate and adaptive immune responses in brains and the severity of lesions. In infected brains T cells response was not restricted to the area with CMV infected cells but diffuse. Future treatments should try to reduce the viral multiplication but also to reduce the inflammatory process.

CMV in Europe

PUBLIC HEALTH IMPACT OF CONGENITAL CYTOMEGALOVIRUS INFECTION IN BELGIUM (ID 035)

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5. Emerging Pathogens Institute and Department of Animal Sciences, University of Florida, Gainesville, FL, USA

Abstract

Congenital cytomegalovirus infection (cCMV) is the commonest non-genetic cause of childhood hearing loss in the post rubella era, a significant cause of neurodevelopmental delay and even a cause of fetal or neonatal death. Unfortunately, there is a continued lack of awareness and prospective randomized controlled studies for perinatal screening and treatment.

Information regarding the epidemiology and public health impact of cCMV infection is vital for evidence-based health policy, for prioritizing the development of much needed prevention and intervention programs, and for evaluating the impact and cost-effectiveness of possible prevention or intervention strategies.

The goal of this study was to assess the public health impact of cCMV in Belgium by using available scientific publications. We performed a systematic review of recent literature on the CMV infection seroprevalence and incidence in women of childbearing age and the cCMV infection incidence in Belgium.

Based on this information the public health impact of cCMV was quantified in terms of Disability-Adjusted Life Years (DALYs), combining disease occurrence and clinical impact in a single number, making it suited to compare different conditions.

The data indicate that cCMV is an important but currently underestimated clinical and public health problem. This congenital infection not only has a significant incidence in Belgium but also induces long-term sequelae from an early age and thus has an important impact on the lives of patients and their families. As a result, this project generated the data needed for the first step in breaking the vicious circle of under recognition and neglect.

CMV in Europe

CONTAMINATION OF SALIVA SWABS DURING A PCR BASED NEONATAL CONGENITAL CYTOMEGALOVIRUS SCREENING PROGRAM: A FREQUENT BUT MANAGEABLE PROBLEM? (ID 033)

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Abstract

Background: Saliva real-time polymerase-chain-reaction (RT-PCR) for the detection of Cytomegalovirus (CMV) in newborns is considered to be a powerful substitute for rapid viral culture showing an enhanced sensitivity combined with a straightforward specimen collection enabling automation at reduced cost. Using PCR techniques, the risk of contamination is an important point of attention.

Material/methods: From 2nd of February 2015 we implemented a universal neonatal CMV screening program in our Belgian hospital. Saliva of the newborn is collected by the pediatrician using Eswab (liquid AMIES, Copan, Italy) on the first postpartum consult and analyzed for CMV DNA by an in house RT-PCR. If positive on saliva, CMV presence is confirmed by performing the same RT-PCR on a urine sample of the newborn. Neonatal PCR results are also compared with CMV serology of the newborn's mother. Data obtained pre conception or in the first trimester of pregnancy and postpartum if the mother was not known to be CMV infected were used. Results were analyzed until 1st of December 2015.

Results:. 2022/2072 (97.6%) of the newborns in our hospital were screened for CMV. 913/2072 (44,1%) of the mothers were found to be CMV seronegative post partum. 43 (2.1%) babies were CMV positive. 10 (0.5%) were considered true cases of congenital CMV, 33 (1.6%) newborns were interpreted to be false positive based on urinary confirmation and serological data of the mother. Contamination most likely occurred during specimen collection and transportation as same results were observed after retesting of these false positive saliva samples. Also blanco internal controls remained negative throughout the study. Cycles of threshold (ct) values of the RT-PCR ranged between 17.8 – 29.7 for true positive and between 30.6 -37.7 for false positive CMV results.

Conclusions: In our universal neonatal CMV screening program we found true positive and contamination rates of respectively 0.5 and 1.6%. RT-PCR CMV ct values on saliva of the newborn allowed to make a clear distinction between true positive CMV results and contamination pointing to large differences in viral load. Emphasis should be placed on actions during the preanalytical phase to prevent CMV contamination.

CMV in Europe

AWARENESS OF CYTOMEGALOVIRUS INFECTION AMONG PREGNANT WOMEN IN GENEVA, SWITZERLAND: A CROSS SECTIONAL STUDY (ID 015)

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Abstract

Background: Cytomegalovirus (CMV) is the most frequent cause of congenital infection and commonly associated with sensorineural deficit. At present, there is neither prophylaxis nor treatment during pregnancy. The objective of this study was to evaluate the level of awareness regarding CMV infection and its consequences in women delivering at the University of Geneva Hospitals (Geneva, Switzerland).

Methods: The study consisted of a validated questionnaire completed by women in the immediate postpartum period.

Results: The questionnaire was completed by 59% (314/528) of delivering women. Only 39% (123/314) knew about CMV and 19.7% (62/314) had received information about preventive measures. Women were more aware about other congenital diseases, such as toxoplasmosis (87%); human immunodeficiency virus (99%); syphilis (85.5%); rubella (92.3%); and group B Streptococcus (63%). Factors associated with CMV awareness were Swiss nationality, high education level, employment in health care or with children, and being followed by an obstetrician. Regarding quality of information, few were aware of the main CMV complications (deafness, 25.2%; mental retardation, 34.5%). Among those informed about CMV, most (74.6%) knew about preventive measures. Among these, 82.5% thought that these were easily applicable.

Conclusions: Most women were unaware of CMV infection and its potential risks during pregnancy. It is crucial to improve CMV information given to pregnant women to prevent the risks for the fetus/newborn.

CMV in the (non) pregnant woman

HUMORAL IMMUNE CORRELATES OF PROTECTION AGAINST SECONDARY CONGENITAL CMV INFECTION (ID 009)

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Abstract

In the setting of primary maternal CMV infection, the risk of transplacental CMV transmission is associated with the development of a poor maternal CMV-specific humoral response. Specifically, women who produce low avidity antibodies with poor neutralization activity against epithelial-tropic variants pose a high risk of transmitting CMV in utero. Whether these same maternal antibody responses protect against congenital CMV transmission in women experiencing CMV reinfection or reactivation of latent virus has not yet been determined. In this study, we used a multivariable logistic regression model to assess the ability of CMV-specific antibody avidity, epithelial neutralizing antibody titers and binding of antibodies to the CMV glycoprotein (pentamer) required for epithelial cell entry to predict the risk of secondary congenital CMV transmission among a cohort of untreated, HIV/CMV co-infected U.S. women and their infants from the Woman and Infants Transmission Study (WITS). Additionally, the magnitude of the maternal antibody binding responses and antibody specificity against a panel of CMV glycoproteins were assessed as secondary immune variables. Multivariable logistic regression was performed with correction for factors previously associated with the risk of congenital CMV infection including: race, maternal age and parity. Results were adjusted for maternal peripheral CD4 T cell counts and HIV virus load and false discovery rate was calculated for the primary immune variables to control for multiple comparisons. Among the primary immune variables, the plasma epithelial-tropic CMV neutralizing response had a borderline significant association with reduced transmission risk with an odds ratio of 0.18 for every log10 increase in neutralizing titer (95% CI 0.03-0.93, uncorrected p value=0.04) which, following correction for multiple comparisons resulted in a non-significant p value (0.12). While not controlled for multiple comparisons, antibody binding to CMV gB linear AD-2 epitope, but not the whole gB glycoprotein, had a borderline significant low odds ratio of 0.72 (95% CI 0.51-1.00; p=0.05) for every 0.5 log10 increase in mean fluorescent intensity. Our data show that maternal plasma neutralization of epithelial-tropic CMV is borderline significant in predicting placental CMV transmission in

seroimmune women. Thus, enhancement of this response via immunization may be an effective means to reduce CMV transmission in CMV-seropositive populations.

CMV in the (non)pregnant woman

PREVENTION AND TREATMENT OF FETAL CYTOMEGALOVIRUS INFECTION WITH CMV-HYPERIMMUNE GLOBULIN: A MULTICENTER STUDY IN MADRID (ID 021)

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Abstract

PREVENTION AND TREATMENT OF FETAL CYTOMEGALOVIRUS INFECTION WITH CMV-HYPERIMMUNE GLOBULIN: A MULTICENTER STUDY IN MADRID

Aims: To investigate the use of cytomegalovirus (CMV) hyperimmune globulin (HIG) in prevention and treatment of CMV fetal infection in Madrid (Spain).

Methods: A retrospective observational study comprising all pregnancies treated with CMV-HIG (2009-2015) in three tertiary hospitals in Madrid was conducted. Investigators offered HIG treatment (200 UI/kg) in pregnancies with a CMV primary infection (prevention group; HIG before amniocentesis) or with fetal infection (treatment group: positive PCR in amniocentesis/cordocentesis). Symptomatic congenital CMV infection at birth was defined as the presence of at least one: abnormal physical exam (petechiae, jaundice, hepatosplenomegaly, neurologic abnormalities), hearing loss, laboratory abnormalities, or abnormal ultrasound or MRI.

Results: During the study period 36 mothers received at least one dose of HIG. Main reasons for consultation were seroconversion (39%), positive IgM and IgG with low avidity test (28%), CMV related findings in fetal ultrasounds (14%), CMV related symptoms in pregnancy (8.3%) and known contact with a CMV infected person (8.3%). Infection was symptomatic in 54.5% of pregnancies.

Median gestational age (g.a) at diagnosis was 20w [IQR=10-25], and at amniocentesis was 21 weeks [20-26]. No severe adverse events of HIG were observed, and median g.a at birth was 38.3 weeks [38-40]. Three children from treatment group were lost to follow up after birth. Prevention group included 17 pregnancies, all with a primary CMV infection. One pregnancy of this group was interrupted due to abnormal cordocentesis and fetal symptoms on follow-up (the necropsy also showed congenital CMV findings). Fetal infection was confirmed in 7/17 (38.5%) patients, and 1/16 (5.9%) was symptomatic at birth (abnormal ultrasound; mild unilateral hearing loss (50 dB), but with good neurodevelopmental outcome at 12 months of age). No other children presented long term sequelae at 12 month of age in the prevention group. Treatment group included 19 pregnancies with positive PCR either in amniotic fluid or fetal blood. One child was born uninfected and asymptomatic after a positive amniotic fluid PCR and 1 dose of HIG. Hearing loss at birth was present in 4/19 (21%), motor impairment in 3/19 (16%) and 9/19 (47%) were symptomatic at birth. At 12 months of age, three children (3/16; 18.8%) in the treatment group presented motor impairment and 4 children (4/16 ; 25%) presented hearing loss. Fetuses with abnormalities in CNS in fetal US before HIG treatment, presented a high risk of long term sequelae (3/3; 100%) compared with those without CNS abnormalities (2/29; 6.7%; p=0.009; OR=77; 95%CI: 3-1954).

Conclusions: In our population CMV-HIG treatment was not associated to relevant adverse events. A high rate of infected fetuses were found in the prevention group. Almost half of children in the treatment group had symptoms at birth. Fetuses without CNS abnormalities in US before HIG treatment presented low risk of long term sequelae. HIG seems not to be useful in fetuses with previous brain abnormalities in US. Randomized controlled trials are needed to close the evidence gap in the HIG treatment of CMV infected fetuses.

CMV in the (non)pregnant woman

LOW INTEREFERON RELATIVE-RESPONSE TO CYTOMEGALOVIRUS IS ASSOSIATED WITH LOW LIKELIHOOD OF INTREUTERINE TRANSMISSION OF THE VIRUS (ID 046)

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Abstract

Introduction: Congenital Cytomegalovirus (CMV) is a very common intrauterine infection which can cause severe mental and hearing impairments. Notably, only 40% of primarily infected women transmit CMV to the fetus. CMV-specific T-cell response has a role in CMV disease but individual immune heterogeneity precludes reliable correlation between measurable T-cells response and intrauterine transmission.

Study aim: To establish a correlation between maternal T cells response and fetal CMV transmission using an individual normalized immune response.

Methods: We analyzed IFN-γ secretion upon whole blood stimulation from primary CMV-infected pregnant women, with either CMV-peptides or PHA-mitogen.

Results: We established a new normalization method of individual IFN-γ response to CMV, by defining the ratio between specific-CMV response and non-specific mitogen response (defined as IFN-γ relative response, RR), aiming to overcome high person-to-person immune variability. We found a unique sub-population of women with low IFN-γ RR, strongly correlated with absence of transmission. IFN-γ RR lower than 1.8% (threshold determined by ROC analysis) reduces the pre-test probability of transmission from 40% to 8%, revealing an unexpected link between low IFN-γ RR and non-transmission.

Conclusion: In pregnant women with primary CMV infection, low IFN-γ RR is associated with low risk of transmission.

CMV in the (non)pregnant woman

CYTOMEGALOVIRUS INFECTION EXACERBATING INFLAMMATORY BOWEL DISEASE IN A WOMAN OF CHILDBEARING AGE. (ID 053)

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Abstract

Background: Cytomegalovirus (CMV), or Human herpesvirus 5, belongs to the family of Beta Herpesviridae. Although the majority of adults will have been infected with CMV by the age of 40 years, 30-50% of women of childbearing age remain susceptible to CMV infection. In chronic inflammatory bowel disease (IBD), the frequency of CMV infection is ranging between 0.62% -3.7%. It is most often a viral reactivation, rather than primary infection.

Observation:

Patient aged of 28 years, gravida 1, para 1, diagnosed in December 2013 for a left RCH on an inaugural thrust. She was treated with a combination of 5-amino-salicylates and corticoids enema. The maintenance therapy with azathioprine (Imurel 2 mg / kg / day) was established later. Four months later, the evolution was marked by an aggravation with increase in mucoid-bloody diarrhea and a rectal syndrome. Clinically, she had a mean condition, 38 ° C fever, tachycardia at 110 beats / minute with abdominal tenderness.

Biologically: inflammatory syndrome with a C-reactive protein (CRP) 107 mg / l and VS 42 mm the first hour, leukocytosis 16000 / mm3 with anemia 8g / dl and hypo-albumin at 26 g /l.

Colonoscopy: Pancolite swollen, little fragile, strewed with a superficials ulcerations and serpigineuses.

The colonic biopsies: a chronicle colitis and intranuclear inclusions with a clear halo surrounding the nucléole and making an owl's eye appearance.

The serology of CMV as well as the research for the deoxyribonucleic Acid (DNA) viral of the CMV by PCR on a sampling of blood was positive.

We conclude the diagnosis of severe acute colitis (CAG) with CMV superinfection

Therapeutically: an antiviral treatment by Ganciclovir 10mg/kg/day was associated with corticoids, during a total duration of 15 days. The clinico-biological evolution was favorable. The patient remained stable after 8 months follow-up

Conclusion : Superinfection CMV in a thrust IBD seems relatively infrequent. Its diagnosis is based on histological evaluation of colonic biopsies. This case presents a double risk : immunosuppression by a possible pregnancy which will add to the IBD, hence the importance of the hygiene practices that may reduce a woman's risk of exposure to CMV and other infections that might pose a risk to her pregnancy and her IBD.

CMV in the (non)pregnant woman

EVALUATION AN ADDED VALUE OF NEWLY AVAILABLE RECOMLINE CMV IgG (AVIDITY) TEST FOR MORE ACCURATE TIMING OF PRIMARY CMV INFECTION (ID 022)

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Abstract

Background. Timing of primary CMV infection in pregnancy is essential as the risks of fetal transmission and consequent medical advice and patient's management are highly dependent on it. The main diagnostic strategy for as correct as possible timing of infection is based on serological testing with the determination of CMV IgG avidity. There are analytical differences between the available CMV IgG avidity tests and the information they provide is rather limited to confirmation of past, mainly > 12 weeks ago, primary CMV. The recent availability of recomLine CMV IgG [Avidity] test (Mikrogen, Germany) using phase-specific antigens in its design would theoretically assure more reliable determination of time of infection. A substantial advantage of this method can be seen in its analytical capacity to determine infections > 24 weeks ago.

Objectives and methodology. To evaluate the additional value of this newly available assay in patient's management, double strategy was followed as this method was evaluated on 1) clinical samples with CMV primary infection > 24 weeks ago as assured by the availability of paired positive CMV IgG samples within this period of time and on 2) clinical samples from pregnant women where more exact timing of primary infection could be helpful in the individual patient's management next to the already routinely performed serology (CMV IgM, CMV IgG, CMV IgG avidity).

Results. Hundred and one samples with CMV primary infection > 24 weeks ago (range 26-129 weeks) were tested with recomLine CMV IgG confirming past infection > 24 weeks ago for 16 (16%) samples; for 58 (57%) samples result of infection >6-8 weeks ago was available; 20 (20%) samples requested additional avidity testing; 4 (4%) samples were reported as positive for CMV IgG and 3: as negative for CMV IgG. For 25 samples (18 with recomLine CMV IgG result infection >6-8 weeks ago and 7 with request for additional avidity determination) recomLine CMV IgG [Avidity] was performed timing infection on 1 (4%) sample > 24 weeks ago; for 19 (76%) samples the result placed infection > 12 weeks ago; for 3 (12%) samples: infection 24 weeks ago to that term in the majority

(83%) of the samples, we could observe benefit of the additional information provided by the use of this method for 4 of the 8 pregnant women. Further analytical evaluation of recomLine CMV IgG [Avidity]as well as its possible added value in the management of individual patients in ongoing.

CMV in the (non)pregnant woman

CMV knowlege and attitudes amongst professionals working in antenatal care in the UK (ID 027)

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Abstract

BACKGROUND: For many years now CMV experts have been calling for health professionals to discuss CMV prevention with pregnant women. Despite this, CMV risk reduction is still not routinely discussed in most countries.

METHODS: CMV Action and St Georges University of London set out to understand more about barriers in the UK. To do this we developed paper and online questionnaires exploring knowledge and concerns amongst three key groups working in antenatal care: midwives, GPs and obstetricians. The survey compared professionals' knowledge of CMV to other infectious diseases and risk factors in pregnancy. It also explored knowledge of prevention measures and attitudes towards prevention.

RESULTS: Views were collected from 79 midwives, 55 GPs and 39 obstetricians working mainly in London and the South East of England.

Many of the professionals surveyed (60% of GPs and 40% of specialists) were actually not confident in their knowledge of CMV. Responses also showed that professionals working in antenatal care in the UK have better factual knowledge of other less common pregnancy issues. However more than 90% of professionals surveyed (and almost 100% of midwives) felt that pregnant women should be given advice about reducing risks of CMV infection. Furthermore when midwives reported having heard of prevention measures, most also reported that they discussed risk reduction with women.

CONCLUSIONS: This survey shows a clear gap between professional willingness to discuss CMV risk reduction and the knowledge they need to do so effectively. The results of this survey are being used by CMV Action in collaboration with academic and healthcare partners to plan and implement a professional development strategy in the UK. This strategy will include insights and recommendations around the practical approach to training professionals and developing supporting

information for women that is needed in the UK to bridge this gap. Elements implemented so far include an e-learning module, a successful grant-funding bid to develop and pilot antenatal resources and a multidisciplinary study day on pregnancy infection.

CMV in the fetus

BLOOD MARKERS IN SEVERLY AND MILD-MODERATELY AFFECTED FESTUSES INFECTED WITH CYTOMEGALOVIRUS (ID 025)

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Abstract

Introduction: In cases of confirmed cytomegalovirus (CMV) infection, certain haematological, biochemical and virological parameters in fetal blood have correlated with neonatal outcome.

Material and methods: Nine-year cross sectional study (2006 –2014). In fetuses with abnormal US/MRI findings and confirmed CMV infection in amniotic fluid, a fetal blood sampling was performed for evaluation of platelet count, beta-2 microglobulin, gamma-glutamyl transferase (GGT), presence or absence of IgM antibody, and DNAaemia. Blood parameter results were compared in severely affected fetuses (severe cerebral abnormalities involving cerebral parenchyma or fetal hydrops) and mild-moderately affected ones (isolated extra-cerebral or mild cerebral abnormalities) diagnosed at sonographic examination, and confirmed by postnatal evaluation or by histological findings after termination of pregnancy (TOP).

Results: Fetal blood sampling was obtained in 19 cases: 14 severely and 5 moderately damaged fetuses. The cordocentesis was performed at a mean gestational age of 29.0 weeks (range 20.4-37.0). Among the severely damaged fetuses there were 13 TOP and one fetal demise. Among the mild-moderately damaged ones there were 3 TOP and 2 alive newborns, one with unilateral hearing loss and one asymptomatic at 18 months of age. A low platelet count (< 100000/µl) and high levels of GGT (≥151 UI/l) were observed in 56% and 69% of fetuses, respectively, and were found more often among the severely damaged fetuses than in the mild-moderately affected ones (62% vs 40% and 89% vs 25%) although the difference was significant only for GGT levels (p=0.38 and p=0.02, respectively). High levels of DNAaemia (>30 000 copies/ml) and beta-2 microglobulin (>11.5 mg/l) were found in 56% and 47% of fetuses, respectively; however, they were observed more often in

mild-moderately damaged, than in severely affected ones (75% vs 50% p=0.58 and 75% vs 36% p=0.28) although again the difference was not significant. In addition, IgM positivity was low and at same proportions in both groups (30% vs 40% p=0.80).

Conclusions: In fetuses with abnormal US/MRI findings and confirmed CMV infection, no significant differences were observed in fetal blood markers with respect to the degree of fetal damage, except for GGT that showed higher levels in the severely damaged.

CMV in the fetus

PRIMARY MATERNAL CYTOMEGALOVIRUS INFECTIONS: HOW ACCYURATE IS FETAL ULTRASOUND TO PREDICT SEQUELAE IN THE OFFSPRING?(ID 008)

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Abstract

Objective: Evaluation of fetal ultrasound accuracy for prediction of sequelae in congenital cytomegalovirus infected fetuses after maternal primary infection.

Study Design: We conducted a prospective observational study between 1996 and 2012. 67 pregnant patients (69 fetuses) were included with serological evidences of maternal primary cytomegalovirus infection and proven vertical transmission to the fetus (positive cytomegalovirus viral load on amniotic fluid or positive viral culture of the neonate). Fetal ultrasound was performed in all patients. Termination of the pregnancy was presented as an option for infected fetuses. Hearing and neurological clinical assessments were performed for all neonates with a cytomegalovirus positive urine sampling.

Results: 67 patients (69 fetuses) with a proven vertical transmission were included in this study, 64 singleton pregnancies and 3 twin pregnancies. 8 fetuses were excluded from the analysis because of insufficient data on the outcomes. Of the remaining 61, Termination of the pregnancy was performed for 26 fetuses. In this group, 11 presented fetal US anomalies. Autopsy confirmed histological evidences of fetal cytomegalovirus infection in all cases. In the 15 fetuses without fetal US anomalies, histological evidence of fetal infection damage was detected in 13 cases. Of the 35 live born infants, 12 had fetal US anomalies suggestive for a congenital infection. Of these 12 infants, 6 had a normal clinical evaluation whereas 6 presented with clinical anomalies from whom 4 cases were considered as severe. In 23 live born children with normal fetal US, 6 infants showed hearing impairments and 2 were diagnosed with mild neurological sequelae.

Conclusion: In a group of maternal primary cytomegalovirus infection acquired in early pregnancy, with proven fetal infection, fetal ultrasound anomalies were detected in 37.7% and were confirmed in autopsy or clinical evaluation after birth in 73.9%. In patients with normal fetal ultrasound evaluation, autopsy or clinical evaluation after birth could detect cytomegalovirus-related anomalies in 55% of the patients.

CMV in the fetus

PRETREATMENT WITH CYTOMEGALOVIRUS HYPERIMMUNE GLOBULIN IS ASSOCIATED WITH PROTECTION AGAINST FETAL LOSS, BUT NOT PLACENTAL TRANSMISSION, IN A RHESUS MODEL OF CONGENITAL CMV TRANSMISSION (ID 040)

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Abstract

The role of maternal antibodies in the protection against congenital CMV transmission and fetal disease in congenitally CMV-infected infants has not yet been clearly defined, leaving open the question of whether CMV vaccine approaches should target antibody responses alone, or include cellular responses. While early studies suggested that CMV hyperimmune globulin (HIG) treatment of mothers following acute CMV infection was effective in reducing congenital CMV transmission and disease, this strategy failed to show efficacy in a larger clinical trial. Thus, to improve our ability to investigate the use of antibody treatment for the prevention of fetal CMV infection, we recently developed a rhesus macaque model of congenital CMV infection. Using this model, we previously demonstrated that maternal CD4+ T cell depletion prior to intravenous rhCMV challenge led to high rates of placental CMV transmission (4 of 4 dams) and fetal loss (3 of 4 dams). To use this model to assess the role of preexisting maternal antibodies in congenital CMV transmission, we tested whether intravenous rhesus CMV (rhCMV) HIG administered before rhCMV challenge could protect against placental CMV transmission and improve fetal outcome in CD4+ T cell depleted dams. Three rhCMV- seronegative pregnant dams were treated with an anti-CD4 T cell depleting antibody at week 7 of gestation, one week prior to rhCMV infection. At week 8 of gestation, the dams received a 100mg/kg intravenous dose of rhCMV HIG followed one hour later by intravenous rhCMV challenge and their outcome was compared to a combined historical and contemporary CD4+ T cell depleted, non-HIG infused group of dams (n = 5). Two of the 3 HIG-treated dams had detectable rhCMV DNA in the amniotic fluid as early as 2 weeks post-infection (mean peak range: 100-14,677 copies/ml) resulting in a 67% congenital CMV transmission rate. All untreated animals also had detectable CMV DNA in the amniotic fluid (mean peak range: 49-580 copes/ml) at week 3 post-infection. Importantly,

none (0 of 3) of the HIG treated dams experienced fetal loss by 6 weeks post infection at the time of fetal harvest, whereas the 4 of 5 untreated dam spontaneously aborted their fetuses by 3 weeks post infection ($p=0.04$, Mantel-Cox log rank test). Furthermore, examination of the tissues of CMV-infected fetuses at 6 weeks gestation revealed the level of CMV DNA in the amniotic fluid reflected the extent of CMV dissemination throughout the fetus. Our results indicate that preexisting polyclonal maternal anti-CMV antibody can prevent fetal demise in nonhuman primates following primary maternal CMV infection, yet does not provide complete protection against congenital CMV transmission.

CMV in the fetus

PRIMARY HCMV INFECTION IN PREGNANCY: FROM CLASSIC DATA TOWARDS METABOLOMICS (ID 029)

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Abstract

Brief introduction. The use of high-dimensional approaches such as transcriptomics (for the study of messenger RNA), proteomics (for proteins) and metabolomics (for metabolites) have been applied on biological samples in order to better understand the physiology or the diseases mechanisms. In particular, metabolomics is the study of the entire range of low molecular weight molecules present in an organ, tissue, or biofluid

Case report. A retrospective cohort study was conducted on 63 pregnant women, 20 of which acquired primary HCMV infection during pregnancy and, subsequently, transmitted the virus to the foetus (transmitters), 20 contracted the infection without transmitting the virus to the foetus (non-transmitters), and 23 healthy pregnant women who underwent amniocentesis for cytogenetic-based diagnosis (controls). The metabolomic profile in amniotic fluid from HCMV-infected and uninfected foetuses was evaluated using GC/MS technique combined with multivariate statistical analysis (PLS-DA) to find out new diagnostic and prognostic biomarkers for congenital HCMV infection. PLS-DA showed a good discrimination for controls vs transmitters and non-transmitters mothers. The most affected metabolism in both cases was the glutamine-glutamate one. PLS-DA analysis was not able to produce a clear separation between transmitters vs non-transmitters. On the contrary, PLS-DA discrimination for asymptomatic vs symptomatic infected foetuses was good. Metabolic pathways analysis revealed the outstanding importance of fatty acids transformations.

Conclusion. Metabolomics represent an intriguing promise for the future development of biomarkers of foetal disease in amniotic fluid following HCMV infection.

CMV in the newborn and beyond

CHILDREN WITH CONGENITAL CYTOMEGALOVIRUS INFECTION FOLLOWING NEGATIVE ANTENAL AMNIOTIC FLUID ANALYSIS HAVE LESS SYMPTOMATIC DISEASE AT BIRTH AND NO LONG TERM SEQUELAE COMPARED WITH THOSE WHO HAD POSITIVE RESULT (ID 006)

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Abstract

Background: Prenatal diagnosis of cytomegalovirus (CMV) infection using amniocentesis is important for both decision making regarding management of pregnancy and planning treatment and follow-up of the newborn. Recently, congenital cytomegalovirus (cCMV) infection despite negative amniotic-fluid analysis for CMV was reported. However, the question of whether this phenomenon represents low sensitivity of the test or late development of fetal infection (after the time of the amniocentesis) was not answered. Moreover, the outcome of these negative prenatal diagnosis cases is yet unanswered.

Objective To compare outcome of infants with cCMV born after negative amniocentesis to those born after positive amniocentesis for CMV, in two different leading international centers for cCMV.

Methods Data of all infants with cCMV infection, who were born after maternal primary infection during pregnancy and were followed in two pediatric centers between 2006 and 2015 were reviewed. Infant outcome after birth of symptomatic vs. asymptomatic disease was compared between infants born after negative amniocentesis (study group) to positive amniocentesis (control group) in a 1:2 ratio.

Results Amniocentesis was performed in 301 pregnancies of our cohort of infants with cCMV and was negative for CMV in 47 (15.6%) and positive in 254 (84.4%). Most (67.4%) of the primary infections took place in early stages of pregnancy (periconceptional or first trimester). Rates of symptomatic cCMV was significantly lower in the study group (negative amniocentesis) than in the control group (positive amniocentesis) (4.3% vs. 25%, $p<0.001$). Hearing impairment at birth occurred less in the study group (2.2% vs. 17.4%, $p=0.012$). On a long term follow up no children in the study group had neurologic sequelae compared to 13 (14.1%) in the control group ($p<0.001$).

Conclusions While negative amniocentesis does not exclude cCMV, infants with cCMV born after negative amniocentesis have very little clinical symptoms and/or imaging findings at birth and very good long term outcome. Our findings support the theory of late development of fetal infection, after the time of the amniocentesis.

CMV in the newborn and beyond

HEARING CONFIGURATION IN CHILDREN WITH cCMV INFECTION AND PROPOSAL OF A FLOW CHART FOR HEARING EVALUATION (ID 043)

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Abstract

Objective: This study had three main goals: (1) to determine the hearing configuration in hearing-impaired children born with a congenital CMV (cCMV) infection, (2) to see whether auditory neuropathy spectrum disorder (ANSD) was present, and (3) to propose a flow chart for the follow-up of hearing in children with cCMV.

Design: Hearing configuration and the presence of ANSD in cCMV infected children was analysed. Selection criteria were: hearing-impaired children with a regular audiometric follow-up for at least 36 months, no other major risk factors for hearing loss, a normal middle-ear status, and an appropriate behavioral response to the given pure-tone stimuli.

Study sample: Out of a cohort of 206 cCMV infected children, 18 hearing-impaired children were selected.

Results: Audiograms of all children showed a flat configuration of SNHL: the slope between octave bands was never greater than 10 decibels. None of the 18 children were found to have ANSD.

Conclusions: Hearing impairment in cCMV infants affected all frequencies equally and ANSD

does not appear to be a feature of cCMV infection. A flow chart for hearing follow-up in children with cCMV infection was suggested in order to provide guidance, improve uniformity in follow-up, and to make results easier to compare.

CMV in the newborn and beyond

Outcome of HCMV Non-Primary HCMV Infection in Pregnancy: Incoming Data from China (ID 005)

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Abstract

Symptomatic HCMV congenital infection in newborns, born by mothers with preexisting antibodies are described from studies done in Alabama (USA) and in Sao Paolo (Brazil). Recent unpublished studies on HCMV seropositive women, done in two hospitals of Shanghai (China), describe HCMV congenital infection of 0,2% and 1,6% in living newborns. All infected infants, diagnosed by PCR from dried blood spots collected at birth, were in this study asymptomatic. This finding is an agreement with the data from the Shandong Province (China) and were optained by Sheila Dollard and Chinese colleagues and presented in Brisbane 2015. These incoming data from China, a country with high prevalence of HCMV antibodies, are in contrast to the data from a high prevalence region (high prevalence population group of young black girls) in the USA and Brazil. Major differences in social behaviour/lifestyle in the study groups of the different regions in the world will be discussed and might explain the different results concerning the outcome in the infected newborns.

CMV in the newborn and beyond

Prevention is better than CMV: A survey of public attitudes towards Cytomegalovirus infection in pregnancy. (ID 014)

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Abstract

Background: Cytomegalovirus (CMV) is the most common congenital infection. 0.5% of those affected die and that 17-20% develop one or more long term neurological sequelae and congenital CMV accounts for up to 5% of cerebral palsy. CMV infection rates in pregnancy can be reduced through the promotion of simple hygiene measures, but there is currently no UK guidance on antenatal prevention of CMV infection and anecdotal evidence suggests awareness among the general public and pregnant women is low.

Methods: ComRes (a UK opinion poll provider) interviewed 2,562 British adults (including 1,008 women aged 18 – 44,) online. Questions were designed to test knowledge of CMV disease and the effect of prevention messages on attitudes. The survey was funded by CMV Action.

Results: Only 10% of 2,562 respondents indicated prior knowledge of CMV disease. The proportion regarding CMV as serious for the unborn baby increased from 73% initially to 88% after reading the information provided. 91% of women aged 18-44 agreed that pregnant women should be given information about preventing CMV infection in pregnancy. 75% agreed congenital CMV is easy to prevent and the majority reported that they would realistically take a range of preventative measures.

Discussion: These results suggest that there is both a need and appetite for knowledge amongst the general public. They imply that CMV prevention messages may result in a change in attitude and willingness to engage in prevention behaviours. There is a need for further evidence to define the relative importance of different prevention measures and how best to present such measures to pregnant women.

CMV in the newborn and beyond

CONGENITAL CMV INFECTION: A SEVERE CASE WIYH UNUSUAL RELAPSING COURSE AND NEW POTENTIAL COMPLICATIONS (ID 038)

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Abstract

Introduction: Congenital cytomegalovirus (CMV) infection is common worldwide. It is the leading cause of nonhereditary sensorineural hearing loss but it can also cause other long-term neurodevelopmental disabilities including cerebral palsy, intellectual disability, vision impairment and seizures. The diagnosis and the management of infants with congenital CMV infection are often challenging due to their extremely heterogeneous presentations and course. We describe a case of congenital CMV infection with unusual presentation and complications.

Case report: A female infant was born by emergency caesarean section at 35-weeks gestation because of prenatal distress. Her mother exams were consistent with a primary CMV infection acquired periconceptionally. Indeed CMV IgM and CMV IgG antibodies were positive and negative respectively during the first trimester of pregnancy, while later a positivization of CMV IgG was highlighted. Neonatal clinical examination showed blueberry muffin syndrome, hepatosplenomegaly, neonatal jaundice and severe hypotonia. Blood examinations revealed pancytopenia, hypertransaminasemia, directed hyperbilirubinemia and hypoglycemia. Transfontanellar brain ultrasound showed bilateral ventriculomegaly and multiple cerebral hemorrhages. Blood tests showed the presence of CMV IgM on neonatal blood. The infection was confirmed by CMV PCR on both the neonatal blood and urine in the first day of life. Treatment with ganciclovir (6 mg/kg/dose IV q12h) was instantly started. During the hospitalization the baby was also transfused with blood and platelets. Because of the persistence of hypoglycemia the patient required high doses of intravenous glucose. Insulin levels were found markedly increased so that a therapy with sandostatin was started. Despite a negative neonatal screening the patient was found to have a mild hypotiroidism treated with levothyroxine. An enlarged thyroid gland was described by ultrasound. After 8 weeks of therapy CMV PCR became negative on blood and ganciclovir was discontinued. At the age of 12 weeks the baby was discharged home with sandostatin and levothyroxine therapy. Nevertheless, 6 weeks after discontinuation of ganciclovir she was taken to the emergency department because of lethargy and weight loss. Blood tests were consistent with a CMV reactivation (positive PCR and IgM) so she was treated with ganciclovir IV (4 weeks) and hyperimmune CMV-IgG. Immunological insights were

conducted showing IgA deficiency < 5 mg/dl (vn 36-165). The baby was later strictly monitored. CMV PCR was persistently negative and since 6 months of age CMV IgG and CMV IgM antibodies were positive and negative respectively. At the latest clinical visit, the patient was 18 months old and she has no longer experienced other episodes of recrudescence of CMV. She was still affected by hypotyroidism and hyperinsulinism. A genetic investigation about hyperinsulinism is actually in course.

Conclusions: The case report is interesting for both the unusual presentation and complications. As for the recrudescence of CMV infection, we hypothesize it was due to reactivation of the congenitally-acquired virus. It could be explained by the combination of a immunodeficiency and the interruption of therapy. The patient received over 8 weeks of appropriately-dosed iv ganciclovir, which we judged to be sufficient at the time. However it is known that severe congenital CMV infections with multiorgan involvement may necessitate longer therapies, lasting even more than 6 months. As for the complications, hyperinsulinism and hypothyroidism have not been described to be associate to CMV infection. Transitory hyperinsulinism is known to be associated with prenatal and perinatal infections, perinatal distress and intrauterine growth retardation, but it usually lasts less than 6 months. Persistent congenital hyperinsulinism may have a genetic cause. Nevertheless, there are no cases reported of persistent congenital hyperinsulinism sustained by congenital infections. Considering the mild hypothyroidism with enlarged thyroid, we hypothesized a cytotoxic effect of CMV on the thyroid gland. This speculation is supported by some literature data that describe the presence of CMV inclusions inside the thyroid of foetus died from congenital CMV infection.

In conclusion, congenital CMV infection may have an unusual presentation and course of the disease. Moreover, a close follow-up is mandatory to prevent further complications.

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CMV in the newborn and beyond

HUMAN CYTOMEGALOVIRUS UL55 (gB) GENOTYPES IN SYMPTOMATIC INFANTS IN ISTANBUL (ID 045)

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Abstract

Introduction;

Human cytomegalovirus (CMV) acquired during intrauterine life or early infancy may result in symptomatic infections which can be complicated with long-term seqelae and even death. A correlation between the severity of clinical manifestations and HCMV genotypes have been suggested by several studies. In this study we investigated the UL55 genotypes in infants with symptomatic HCMV infection.

Patients and Methods;

Twenty six symptomatic infants infections who were referred to our laboratory for CMV DNA test and yielded positive results between 2011-2015 were included in the study. UL55 region was amplified by in-house nested PCR from 29 plasma and 2 urine samples. One infant had both plasma and urine samples and 1 infant had only an urine sample available. In addition 8 of the 29 plasma samples were obtained from 4 infants on two different occasions. PCR products were purified and sequenced bi-directionally by dideoksi chain thermination method. The sequeces were edited using SeqMan software. Multiple aligment was performed using ClustalW program. Phylogenetic analysis was conducted using MEGA6 software and the phylogenetic tree was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Kimura 2-parameter method and bootstrap test (1000 replicates) was used for interpretation. The analysis involved 40 nucleotide sequences, 31 sequences obtained form our patient group and 9 sequences downloded from gene bank as reference sequences of different genotypes (Merlin;GB1, M60927;GB1, M60929;GB1, M60931;GB2, AD169;GB2, AC146907;GB3, M85228;GB3, M60926;GB4, AF043721;GB5).

Results and Conclusion(s);

The mean age of the study population was 8+5 months with a sex ratio of 1:1. The distribution of the genotypes are given in the table. Nearly half of the infants harboured UL55 genotype 1, which was

suggested to be linked with asymptomatic HCMV infection in infants. No mixed genotypes were detected and repeated samples yielded the same genotypes.

Table: The distrubution of the CMV genotypes in 26 infants.

Genotype	n	%
1	14	53,85%
2	6	23,08%
3	4	15,38%
4	2	7,69%
5	0	0,00%
Total	26	100,00%

CMV in the newborn and beyond

CEREBROSPINAL FLUID FINDINGS IN CYTOMEGALOVIRUS CONGENITALLY INFECTED ASYMPTOMATIC INFANTS (ID 044)

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Abstract

BACKGROUND: Cytomegalovirus (CMV) congenitally infected asymptomatic infants may develop neurological sequelae (sensorineural hearing loss, microcephaly, motor defects, chorioretinitis) in 10% of cases. CMV detection in the cerebrospinal fluid (CSF) by means of polymerase chain reaction (PCR) or viral culture, and CSF indices (WBCs and proteins) over the upper cut-points for the age, have been variably proposed in order to identify brain infection in congenitally infected infants; nonetheless, CSF findings in CMV congenitally infected asymptomatic infants are currently unknown. METHODS: Retrospective review of cases of asymptomatic congenital CMV infection diagnosed from 2009-2015 at the Neonatology Unit of the University of Rome “La Sapienza”, Italy, who underwent lumbar puncture (LP) for CMV DNA PCR search and CSF indices evaluation . RESULTS: twenty-one congenitally infected asymptomatic infants [20 maternal primary infections (1 prenatal, 10 first trimester, and 9 second trimester infections) and 1 undefined infection] had a LP performed. 2/21 (9.5%) cases were excluded due to the bloody appearance of the CSF. CMV DNA search was available in 19/19 (100%) cases, while CSF indices were obtained in 17/19 cases (89.5%) due to insufficient sampling in two cases. Correction factors for CSF indices were applied in 4/17 (23.5%) traumatic LP (RBCs >500/mm3). Elected CSF indices cut-off, according to the existing literature, were WBCs >19/mm3 and proteins > 1150 mg/L. 2/19 (10.5%) infants had a positive CMV DNA search (2.835.371 copies/ml in a first trimester infection and 1404 copies/ml in a second trimester infection) on two non-traumatic samples; WBCs, but not proteins, were elevated in one of these two cases. Of note, both these infants had a brain MRI performed with negative results. 9/17 (52.9%) asymptomatic infants had a WBC count >19 cells/mm3 (3 traumatic samples) and 2/17 (11.8%) had CSF proteins > 1150 mg/L. Overall, 10/17 (58.8%) infants had abnormal CSF indices

when compared to the currently existing cut-off. CONCLUSIONS: 1) CSF CMV DNA search identified brain infection in 10.5% of otherwise asymptomatic infants. 2) Currently used CSF indices cut-off, derived from studies of non-infected infants, do not discriminate brain infection (as detected by means of PCR) in CMV congenitally infected asymptomatic infants. Reference intervals for CSF indices, based on a large population of CMV congenitally infected asymptomatic infants, are needed to define abnormal values in this population. 3) Larger studies with accurate neurodevelopmental follow-up are necessary in order to evaluate the prognostic meaning of these findings and the need, if any, of antiviral therapy.

CMV in the newborn and beyond

Infants diagnosed with congenital CMV after NHS refer: a wide variety of signs in congenitally infected infants without clinical signs at birth (ID 024)

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Abstract

Background

The majority of infants with congenital CMV (cCMV) with long term sequelae (hearing loss, visual impairment, developmental delay) are born without clinically apparent disease in the neonatal period. If CMV diagnostics is only performed in symptomatic infants many infants at risk of developing sequelae will not be diagnosed. Data in the CONCERT study gives more insight into sequelae in infants without clinically apparent disease, referred after neonatal hearing screening.

Methods

Parents of infants who failed neonatal hearing screening (NHS) in the Netherlands were invited to participate in the CONCERT study (NCT02005822). Dried blood spots, obtained within 5 days of birth, were tested for CMV. A physical examination was performed in children with cCMV at the age of 2-3 months. Audiological examination was performed before the age of 2 months. Brain ultrasound was performed upon consulting a Pediatrician.

Results

1081 infants who failed NHS were tested for cCMV, and 47 of these (4,3%) tested positive. At the first hearing evaluation bilateral hearing loss was confirmed in 21, unilateral hearing loss in 21 and 5 infants had normal hearing. Physical examination revealed 6 infants with a head circumference ≤-2 SD. Laboratory findings were complete for 23 infants and revealed 5 infants with neutrophil count <1,0 x10^9/L (all with normal total leucocyte count) and 3 infants with elevated liver transaminases (ASAT ≥89 U/L ; ALAT ≥60 U/L). None of these infants required additional treatment. With brain ultrasound performed in 30 infants, abnormalities were found in 22 infants. MRI examination was

performed in 14 infants and revealed abnormalities in 6 infants ranging from mild defects to polymicrogyria.

Conclusions

These data emphasize the risk for developing hearing loss without apparent clinical disease. Also in many infants abnormalities were found with brain ultrasound and MRI examination. Considering that infants diagnosed with congenital CMV receive additional care (extra hearing evaluations, brain ultrasound, medical support from a Pediatrician and ophthalmologic evaluation) CMV diagnostics should be offered to all infants with a NHS refer.

CMV in the newborn and beyond

OUTCOME OF CONGENITAL CMV SENSORINEURAL HEARING LOSS IMPLANTATIONS: CEREBRAL ANOMALIES (ID 032)

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Abstract

Introduction: Congenital CMV infection is responsible of 20 to 30% of all hearing losses. Some of these will require cochlear implantation. The aim of this study is to evaluate the outcome of implanted children with or without cerebral lesions after congenital CMV infection

Method: Retrospective study concerning 333 patients who received a cochlear implant between 1998 and 2013. Twenty six patients were identified as being profoundly deaf after congenital CMV infection: aetiologic diagnosis was confirmed in 16 cases by PCR, 7 had suggestive MRI cerebral images, 3 cases had suggestive medical history in favour of congenital CMV infection. Language level was assessed and the APCEI and K index were calculated for each.

Results: The mean age of implantation was 3.7 year (1.3 year to 16). Eight had a history or prematurity and were symptomatic at birth. All but five had cerebral lesions identified on the MRI: periventricular white matter anomalies, ventricular dilations, polymicrogyria, calcifications. Four presented with neural sequelae as hypotonia and 3 developed psychiatric disorders. Three groups could be identified according to outcome: a positive group (12 patients) for which results were close to normal patients at 4 years; an intermediate group (3 patients) of slow progressing patients; and an unfavourable group (3 patients). The follow up for 8 patients was shorter than 2 years, therefore too short to evaluate the outcome: however, at 1 year post-surgery, 3 had a K index at 5, 4 had a K=2. In the unfavourable group, psychiatric disorders led to explantation or abandon of the implant for 2 patients and the last patient had little benefit from the cochlear implant. No patient with normal MRI had a $K \leq 3$, however, 11/15 patients with abnormal MRI had a $K \geq 4$ at 4-year follow-up.

Conclusion: For most CMV congenitally infected children with profound hearing loss, cochlear implantation show similar results to children implanted for other aetiologies. However, MRI cerebral anomalies should be systematically assessed : expectations of cochlear implant benefits should be pondered according to their severity.

CMV in the newborn and beyond

Risk factors of maternal-fetal mixed herpes infection in infants children (ID 051)

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Abstract

Actuality: maternal-fetal herpes infection is an infection commonly spread through opportune infant infectious pathologies. The highest incidence of herpes infections is due to several factors: epidemiological and evolutionary character (evolving latent-persistent multiple mechanisms and ways of transmission, purchaseboth subclinical forms primary and secondary infection)immune system status in pregnant women, fetus and neonate. The infection has tended to increase due to late diagnosis and lack of antenatal screening. The clinical manifestations of the infection depend on the following factors:

1. The term of pregnancy at which infection has occurred.

2. Virulence of the pathogen, the ways of penetration.

3. Primo-infection or recurrence of infection during pregnancy.

Purpose:

1. To find antenatal and postnatal risk criteria of maternal-fetal infection.

2. To diagnose herpes infection in infants.

Materials and Methods: The study group included 120 children were hospitalized in SCMC No.1, during 6 months 2014. Patients were divided into 2 groups:

-1 to 6 months 45 (37.5%);

-6 to 12 months 75 (62.5%);

The diagnosis was given by PCR and enzyme immunoassay, biological materials: plasma and urine.

Results:

1. Antenatal risk factors: the presence of herpes infection in the mother, miscarriages (spontaneous abortion),stagnated pregnancy, born dead, aprevious birth of children with disabilities or who died soon after birth.A history of infertility- were detected in all pregnant interviewed women;
2. Postnatal risk factors: microcephaly, calcined, pseudocysts in the brain were detected in 56.5% of children in the first group and 43.5% in the second group;
3. Malformations of organs found in 32% of cases.
4. Prolonged neonatal jaundice was detected in 52% of both groups of children, hepatosplenomegaly was present in 100% of cases.
5. DNA CMV, HSV type 1.2 in the urine was detected in 51.8% of children in the first group and 48.2% in the second group.
6. The high level more than 3 times of the specific CMV end HSV was detected in 100% of cases.

Conclusions:

1. Maternal-fetal infection is a major risk of infection to the fetus.
2. Risk factors can be used for early detection of infection with depth investigation and prognosis the evolution of the infection.

Growing up with congenital CMV

TWO-YEAR OUTCOMES FOR CHILDREN WITH CONGENITAL CYTOMEGALOVIRUS INFECTION (ID 037)

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Abstract

Cytomegalovirus (CMV) is the most important cause of congenital viral infections in the world. It is known that congenital CMV infection rates are high in populations with high CMV seroprevalences. Determination of symptomatic congenital CMV infection in babies with seroimmune mothers increases the importance of this topic especially in societies with high seropositivity. In our previous studies it has been found that CMV seroprevalence and congenital CMV infection prevalence were 93.6% and %0.87, respectively, in Antalya, Turkey. The aim of the study was the examination of physical, mental and motor development and hearing status of the children who had congenital CMV infection in Antalya, Turkey.

In a prospective study 10 children with congenital cytomegalovirus infection born between May 2012 and December 2013 were called back around their second age for evaluation for their physical, mental and motor growth and hearing status in Antalya, Turkey. Out of ten infants eight have agreed to come to the hospital. Among these infants; two had prematurity and low birth weight, one had microcephaly, prematurity and low birth weight and one had low birth weight at birth and all of them had passed Newborn Hearing Screening Test.

For eight cases; full audiological examination and assessment were carried out, general development was assessed and urine CMV DNA was measured by a quantitative real time PCR (COBAS AmpliPrep/COBAS TaqMan) test. In addition complete blood counts, liver function tests were analysed. For hearing status; transient otoacostic emissions (TOAE) were used to screen all infants, followed by automated auditory brainstem response (ABR) in those who did not pass TOAE

CMV DNA was still positive in urine samples of all infants. A male infant with microcephaly, prematurity and low birth weight at birth had moderate mental, motor and growth retardation and strabismus. His MRI scan showed that lateral ventricles dilatation and mineralization in the brain. A female infant's who was asymptomatic at birth, left ear did not pass TOAE testing, although she had passed the initial hearing screening test using TOAE for both ears at the newborn period. Then ABR test was performed, and revealed a 90 dB sensorineural hearing loss on her left ear. Slightly increased AST levels were found in four infants.

In conclusion; congenital CMV infections cause important sequelas and hearing loss in highly seroimmune populations.

Growing up with congenital CMV

EVALUATION OF VESTIBULAR DISORDERS IN CONGENITAL CYTOMEGALOVIRUS INFECTION (ID 012)

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Abstract

Introduction : Congenital cytomegalovirus (CMV) infection is one of the leading cause of congenital hearing loss. Histological studies indicate as much vestibular damages as cochlear. This prospective, single-center, transversal study aimed to assess the prevalence of vestibular disorders secondary to congenital CMV infection.

Case report : 24 patients followed for a congenital CMV infection had a vestibular assessment, the average age was 35 months. 33,3% had a clinical expression of the CMV infection during the evaluation ; 20,8% had hearing loss. 34,8% had a vestibular disorder. 80% of children with hearing impairment and 22,5% of children with normal hearing had a vestibular disorder. 57,1% of patients with symptoms of the congenital CMV infection at birth had vestibular impairments, and 25% of children asymptomatic at birth. At the examination, 75% of children symptomatic and 13,3% of those asymptomatic had vestibular impairments. Vestibular impairments are more severe in symptomatic children at birth or becoming, and are correlated with delayed development, particularly in a context of joint encephalopathy. At 4 months, head control was acquired for 93,3% of children with normal vestibular fonction, versus 50% in case of vestibular impairment. The median age of unaided walking was 18 months for children with vestibular impairments, 12 months when vestibular function was normal.

Conclusion : vestibular disorders in congenital CMV infection are more frequent than hearing impairments ; they are more severe in symptomatic children at birth or becoming, and are correlated with delayed postural development, particularly in a context of joint encephalopathy. In asymptomatic children, vestibular disorders are less common, unilateral, and have no impact. Vestibular assessment should be performed in all children with clinical expression of congenital CMV infection between 6 and 12 months, to guide their psychomotor education.

Growing up with congenital CMV

Symptoms and sequelae attributable to congenital Cytomegalovirus infection (ID 030)

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Abstract

Introduction

Congenital cytomegalovirus infection (cCMV) may lead to symptoms at birth and long term sequelae. Limited data on long term sequelae are available, particularly in infants who are asymptomatic at birth. Information comparing symptoms and sequelae with a control group is seldom provided.

Aim and methods

A nation-wide retrospective cohort study was designed to assess long term consequences of cCMV up to the age of six years in the Netherlands. cCMV was diagnosed using polymerase chain reaction on dried blood spots, collected for neonatal screening purposes. Data on 133 cCMV positive children and 274 cCMV negative controls (matched for age, gender and region), were collected from general physicians and other health care providers. Symptoms that could possibly be related to cCMV in the neonatal period and permanent long term consequences, including hearing, visual, neurological, motor, cognitive and speech-language impairment, were compared.

Preliminary Results

In the cCMV positive group 26 (19.6%) children showed cCMV-related symptoms at birth, whereas in the cCMV negative group 34 (12.5%) children had similar symptoms. This indicates a risk difference between the cCMV positive and cCMV negative groups of 7.1% (95% CI: -0.7 - 14.9).

Overall 34 (25.6%) cCMV positive and 29 (10.6%) cCMV negative children had one or more long term impairment (risk difference: 15.0%, 95% CI: 6.7 - 23.2). The highest risk difference for

impairments (48.9%, 95% CI: 27.6 - 70.1) was observed in children with cCMV-related symptoms at birth, when cCMV positive (57.7%) and cCMV negative children (8.8%) were compared. In children without symptoms at birth, only a small non-significant risk difference for impairments was observed (6.9%, 95% CI: -1.4 - 15.1) between the cCMV positive (17.8%) and negative (10.9%) groups.

Conclusion

Symptoms at birth that have previously been attributed to cCMV also occurred rather frequently among cCMV negative children (12.5%). The risk difference of 7% for these symptoms is significantly lower than the generally accepted assumption that 10 to 15% of cCMV children are symptomatic at birth.

Long term impairments were more common in the cCMV positive than the cCMV negative children. This difference in impairments was statistically non-significant among children who were asymptomatic at birth. However, in the group of children who were symptomatic at birth there was a large difference in impairments between the cCMV positive and cCMV negative groups.

These findings demonstrate the need to reinterpret the role congenital CMV infection plays in causing symptoms at birth as well as long term sequelae.

New therapeutic approaches

PHARMAKOKINETIC CHARACTERISTICS OF HYPERIMMUNE GLOBULIN TREATMENT IN PREGNANCY: IMPACT ON IGG AVIDITY MATURATION AND NEUTRALISATION (ID 034)

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Abstract

a) Brief introduction. CMV seronegative pregnant mothers in low prevalence countries (<70% CMV IgG positive) with exposure to CMV shedding infants below three years are under high risk to acquire via infant-to-mother-transmission a CMV primary infection. In contrast to viral reinfection or reactivation during pregnancy, the maternofetal transmission rate is quite high in the range of 30-50%. About 10-15% of prenatally infected infants acquire long term sequelae like hearing loss. For prevention of maternofetal CMV transmission, a CMV specific hyperimmune globulin (HIG) preparation has been used in several studies (Nigro et al., 2005; Buxmann et al., 2012). In contrast to the initial nonrandomized study by Nigro, a recent randomized, placebo-controlled clinical trial revealed no significant reduction of CMV transmission, 2014). However, a 14% reduction of the numbers of congenitally CMV-infected newborns was observed (Revello et al., 2014).

b) Case report. In order to investigate the influence of HIG administration on the CMV IgG avidity maturation, the increase of IgG levels, the recombinant antigen specific antibody response, and on the HIG-induced neutralisation capacity using epithelial cells and a clinical viral isolate from amnion fluid, we performed pharmacokinetic analysis of an index case with HIG treatment cycles via monthly doses of 100PEIU/kg/dose of HIG. We found strongly fluctuating levels of CMV IgG, recombinant IgG avidity against IE1/p150/CM2 antigens and CMV neutralisation titers (NT) and NT50 values against ARPE-19 cells in vitro (Hamprecht et al., 2014). Based on this observation we changed the administration regimen on biweekly intervals of HIG treatment cycles.

The pharmacokinetic analysis based on defined time points of blood samples gained during HIG treatment cycles (-15min, +30min, +2h, +7d, +14d) included up to now 15 women. In all these women

we found individual kinetics of fluctuating CMV-specific antibodies confirming our initial observation. Interestingly, we observed interindividual differences in the response to HIG depending on the initial CMV IgG level and the gestational age at beginning of HIG treatment.

c) Conclusion. The maternal antibody response to biweekly administrated HIG doses seems to result in a more sustained CMV neutralisation capacity than the usual 4 week-intervals. Additionally, an increase of CMV-specific IgG can be observed during sequential treatment cycles, while the monthly HIG regimen goes down to baseline levels for all CMV antibody parameters under investigation. The shortening of the initial HIG treatment schedule of NIgro et al. 2005 might have important implications for the HIG efficacy to prevent maternofetal CMV transmission. Primary endpoint of our clinical study arm is the prevention of maternofetal CMV transmission, documented by amniocentesis. Results will be evaluated still in 2016.

New therapeutic approaches

The broad-spectrum antiinfective drug artesunate and novel optimized derivatives interfere with the canonical nuclear factor kappa B (NF-kB) pathway by targeting RelA/p65 (ID 019)

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Abstract

A novel approach focuses on HCMV inhibitors derived from natural resources or semi-synthetic derivatives like the antimalaria and broad antiinfective drug artesunate (ART). Thereby, an improvement of therapeutic options may be provided, such as a high oral bioavailability and low levels of side-effects. Currently, artemisinins are recommended by the WHO as first-line therapy for pregnant women with severe malaria during the second and third trimester. In addition to antimalaria activity, ART possesses strong and broad antiviral activities and is particularly efficacious against HCMV. In this study, novel ART-derived compounds including dimers and trimers were synthesized and analyzed for antiviral activity and the responsible underlying molecular mechanism. Importantly, some derivatives showed substantial improvements over the parental drug. The overall evaluation of antiviral and mechanistic activities led to the following statements: (i) ART exerts broad antiviral activity towards human and animal herpesviruses, (ii) no induction of ART-resistant HCMV mutants occurred in vitro, (iii) chemically modified derivatives of ART showed strongly enhanced anti-HCMV efficacy, (iv) NF-kB reporter constructs, upregulated during HCMV replication, could be partially blocked by ART treatment, (v) ART activity analyzed in stable reporter cell clones indicated an inhibition of stimulated NF-kB but not CREB pathway, (vi) solid-phase immobilized ART was able to bind to NF-kB RelA/p65, and (vii) synthetic peptides of NF-kB RelA/p65 represented candidates of ART binding as analyzed by in silico docking and mass spectrometry. These novel findings open new prospects for the future medical use of ART and ART-related drug candidates.

Primary and non-primary infection in the mother

HCMV-specific antibodies and HCMV-specific T-cell response may help date primary infection in pregnancy (ID 052)

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Abstract

Background: Dating HCMV primary infection during pregnancy is critical to discriminate between primary and remote infections and to define gestational age at time of infection.

Methods: We studied B and T-cell response to HCMV in 53 pregnant women experiencing primary HCMV infection. We analyzed the following B cell parameters: a) conventional assays (IgG and IgM to HCMV whole antigen and IgG avidity index); b) antibodies neutralizing infection of epithelial and fibroblast cells; c) ELISA IgG antibodies to HCMV glycoprotein complexes gH/gL/UL128L, gH/gL/gO and gB. As for T-cell response, we analyzed CD4+ and CD8+ IFN-gamma and IL-2 production and lymphoproliferative response (LPR). As controls, we examined 33 pregnant women who experienced infection at least 5 years before (remote infection).

Results: IgG antibodies to gB, gH/gL/pU128L, and antibodies neutralizing infection of fibroblasts appeared early (within 2-3 weeks of infection) and increased rapidly, whereas antibodies to gH/gL/gO appeared later (>30 days) and increased slowly. Novel and conventional assays allowed the definition of an algorithm indicating the onset of infection within time intervals of 1-2 months. HCMV-specific CD4+ and CD8+ T-cells appeared early (within15 days) after infection. CD4+ T-cells increased reaching values similar to remote infection after 90 days. CD8+ T-cells expanded in the first 60 days, persisting at levels significantly higher than those observed in remote infection for the entire follow-up (2 years), suggesting a subsequent contraction phase. LPR remained low or undetectable for the first 6 months while IL-2 production remained low for the 2-year period examined. Results of IL-2 production and LPR were useful to discriminate infections occurring within 6 months, within 6-24 months, or later.

Conclusions: Determination of antibodies specific for HCMV glycoprotein complexes and IL-2 production and LPR by HCMV-specific T-cells may help date infection and differentiate primary and remote infection.

Primary and non-primary infection in the mother

CONGENITAL CMV INFECTION AND TIMING OF MATERNAL PRIMARY INFECTION (ID 056)

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Abstract

Background: CMV infection is the most common congenital infection with potentially severe long term sequelae. Majority of the infected infants are asymptomatic as newborn. Only about 10% of the infected babies have typical signs and symptoms suggestive for congenital CMV. The long term sequelae are more common in the group of symptomatic babies. Fetal infection may occur after maternal primary or non-primary infection during pregnancy. Non-primary infection means either re-activation of a latent infection or a new infection with a new CMV strain. We have studied whether the proportion of maternal primary / non-primary infection during pregnancy was different in the babies with symptomatic and asymptomatic infection.

Methods: The first cohort consisted of 29 symptomatic babies diagnosed congenital CMV on clinical basis. We executed a database search on hospital records in all five University Hospitals in Finland to find out the infants with diagnosis P35.1 (congenital CMV infection). Only symptomatic infants were included in the analysis. The second cohort consisted of 41 asymptomatic infants diagnosed in

a screening study of 20 000 infants born 2012 – 2015. As a screening tool we used a saliva CMV PCR test. The preserved maternal serum samples taken in the early pregnancy (H10-12) were analyzed for CMV antibodies to determine the timing of maternal primary infection. The infection was considered to be non-primary, if the mother had IgG and no IgM in the early pregnancy samples. Early pregnancy samples with positive IgM and low avidity IgG were considered to mean primary infection in the 1st trimester or near conception.

Results: The timing of maternal primary infection did not differ in the cohorts. Maternal non-primary infections accounted for 54 % of congenital infections in both symptomatic and asymptomatic infants. Of the primary infections during pregnancy, in 7/12 (58%) of the symptomatic cases and in 5/19 (26%) of the asymptomatic cases, the maternal infection occurred in the first trimester or near conception (p=0.130).

Conclusions: Non-primary infections play an important role in both symptomatic and asymptomatic congenital CMV infection. This underlines the fact, that in purpose of preventing congenital CMV infection, we should not focus only on seronegative mothers.

CMV in the (non) pregnant women

INTERACTION BETWEEN SOLUBLE HLA-G EXPRESSION AND HCMV INFECTION DURING PREGNANCY (117)

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Human cytomegalovirus (HCMV) infection is the leading cause of congenital viral infection and brain disease in children in developed countries. The use of advanced serological and virological maternal tests allow to identify pregnant women who are at higher risk of transmitting HCMV to their fetus. Today, the fetal compartment can be only studied by amniocentesis and ultrasound examination for the diagnosis and prognosis of CMV infection. Since amniocentesis is an invasive procedure and positive results of amniotic fluid (AF) tests do not discriminate between infected fetuses and compromised fetuses, researchers continue to work on the prognosis factors for the HCMV disease.
In order to improve the identification of i) pregnant women who transmit the virus to their fetus and ii) HCMV-infected and compromised fetuses, we studied the expression of soluble isoform of the human leukocyte antigen class I "non-classical" (sHLA-G) during HCMV infection in maternal blood and amniotic fluid samples. HLA-G antigen is a tolerogenic molecule and is modified by HCMV infection, with possible functional consequences in pregnancy immuno-regulation.
We describe the interim analysis of a clinical prospective trial that is enrolling 400 pregnant women suspected, at routine HCMV testing, to have active HCMV infection. Here, we report the data obtained from a first cohort (69%) of 276 pregnant women. In particular, the sHLA-G levels were evaluated in the plasma samples of 91 pregnant women with primary HCMV infection, 57 with non-primary, 103 with past infection, and 25 HCMV-uninfected.
We found that the mean levels of sHLA-G in pregnant women with primary infection were significantly higher in comparison with the other mentioned groups (42.32 ng/ml vs. 9.07 ng/ml, 11.87 ng/ml, 7.14 ng/ml, respectively, $p<0.001$; Student's t-test). When we analyzed the levels of sHLA-G in plasma samples from primarily infected pregnant, considering transmitter and non-transmitter mothers, we did not find any statistical correlation ($p=0.72$; Student's t-test).
Moreover, we analyzed 37 AF samples collected during amniocentesis (20-21 weeks gestation) from pregnant women with primary HCMV infection. The mean levels of sHLA-G were significantly higher in 10 samples from infected fetuses (49.19 ng/ml) than in 27 uninfected fetuses (19.03 ng/ml) ($p <0.001$; Student's t-test).

Our preliminary results suggest that sHLA-G detected in plasma and AF samples might be an additional biomarker of HCMV infection that could be considered in combination with currently used serological and virological markers. The probability that the independent evaluation of sHLA-G to recognize the risk of developing primary maternal HCMV infection and fetal HCMV infection is not 100%.


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A FIRST-IN-HUMAN STUDY TO ASSESS THE SAFETY AND PHARMACOKINETICS OF MONOCLONAL ANTIBODIES AGAINST HUMAN CYTOMEGALOVIRUS IN HEALTHY VOLUNTEERS **(Novartis)**

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Background: Human cytomegalovirus (HCMV) can cause significant disease in immunocompromised patients and treatment options are limited by toxicities. CSJ148 is a combination of two anti-HCMV human monoclonal antibodies (LJP538 and LJP539) that inhibits HCMV infection of various cell lines *in vitro*.
Methods: Here, we evaluated the safety, tolerability, and pharmacokinetics of a single intravenous dose of LJP538 or LJP539 or their combination in healthy volunteers.
Results: Adverse events and laboratory abnormalities occurred sporadically with similar incidence between antibody and placebo groups and without any apparent relationship to dose. No subject who received antibody developed a hypersensitivity, infusion-related reaction or anti-drug antibodies. Following intravenous administration, both LJP538 and LJP539 demonstrated typical human IgG1 pharmacokinetic properties, with slow clearances, limited volumes of distribution, and long terminal half-lives. Pharmacokinetic parameters were linear and dose-proportional for both antibodies across the 50-fold range of doses evaluated in the study. There was no apparent impact on pharmacokinetics when the antibodies were administered alone or in combination.
Conclusions: CSJ148 and the individual monoclonal antibodies were safe and well tolerated, with pharmacokinetics as expected for human immunoglobulin.

IN VITRO CHARACTERIZATION OF HUMAN CYTOMEGALOVIRUS-TARGETING THERAPEUTIC MONOCLONAL ANTIBODIES LJP538 AND LJP539 **(Novartis)**

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Background: Human cytomegalovirus (HCMV) infection is usually benign in healthy individuals, but can cause life-threatening disease for those with immature or compromised immune systems. Approved drugs available to treat HCMV disease, including ganciclovir, cidofovir, and foscarnet, have significant toxicities that limit their use in certain patient populations. LJP538 and LJP539 are human monoclonal antibodies that are under evaluation as immunoglobulin therapeutics. The antibodies target glycoproteins gB and the gH/gL/UL128/UL130/UL131a pentameric complex, respectively.
Methods: Here we present an *in vitro* characterization of these antibodies.
Results: We confirm that they block viral infection and syncytia formation in culture. We show that LJP538 and LJP539 are more potent than a marketed immunoglobulin at inhibiting HCMV infection of various cell lines relevant to pathogenesis. Furthermore, we show that LJP538 and LJP539 are active against a panel of clinical isolates *in vitro* and demonstrate minor to moderate synergy in combination studies.
Conclusions: Taken together, these data support the use of LJP538 and LJP539 in combination for HCMV clinical trials in susceptible patient populations.

Prevention

OPTIMIZED ENZYME LINKED IMMUNOSORBEN ASSAY FOR DETECTING CYTOMEGALOVIRUS INFECTION IN SUBJECTS VACCINATED WITH A CYTOMEGALOVIRUS GLYCOPROTEIN B (CMV GB) VACCINE **(Sanofi)**

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Abstract

The glycoprotein B of CMV is one of the vaccine candidates proposed to prevent CMV infection and is also present in the commercial ELISA kits used to diagnose CMV infection. In gB-based clinical trials, to differentiate antibodies induced by infection and by the vaccine, sera from vaccinated subjects have thus first to be depleted of anti-gB antibodies before measuring anti-CMV antibodies with a commercially available ELISA kit. Here, we developed an enzyme-linked immunosorbent assay (ELISA) that eliminates the need for gB pre-absorption. As possible capture antigens, we evaluated antigenic regions of UL83/pp65, UL99/pp28, UL44/pp52, UL80a/pp38, UL57, and UL32/pp150. An IgG ELISA using a UL32/pp150 [862-1048] capture peptide was the most specific (93.7%) and sensitive (96.4%) for detecting CMV in sera. The ELISA successfully detected CMV infection in 100% sera of subjects who had been vaccinated with a gB vaccine but who had later been infected with CMV. In four of these cases, the ELISA detected CMV infection before the gB pre-absorption assay. This IgG ELISA was shown to be linear, reproducible and specific and can be used in place of a gB pre-absorption assay for the sensitive and specific detection of CMV infection in gB-vaccinated individuals.