CHAPTER 4.2

QUANTITATIVE MR IMAGING AND SPECTROSCOPY IN CONGENITAL CYTOMEGALOVIRUS INFECTION AND PERIVENTRICULAR LEUKOMALACIA SUGGESTS A COMPARABLE NEUROPATHOLOGIC SUBSTRATE OF THE CEREBRAL WHITE MATTER LESIONS

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ABSTRACT

Congenital cytomegalovirus (CMV) infection and periventricular leukomalacia (PVL) both lead to static cerebral white matter lesions. In contrast to PVL, the neuropathologic substrate of these lesions in congenital CMV is not clear. By comparing changes in quantitative Magnetic Resonance (MR) parameters and MR spectroscopy metabolite concentrations we wanted to determine whether the nature of the white matter pathology in congenital CMV infection could be similar to the known pathology of PVL. Diffusion parameters apparent diffusion coefficient (ADC) and fractional anisotropy (FA), magnetization transfer ratio (MTR) and MR spectroscopy concentrations were studied in white matter lesions in five patients with a congenital CMV infection and six patients with PVL. In both groups ADC values were increased, FA and MTR values were reduced, concentrations of total N-acetyl-aspartate and choline-containing compounds were reduced; and *myo*-inositol concentrations were slightly increased. No differences were found between the two groups, suggesting that the pathology of the white matter lesions in congenital CMV infections is similar to that of PVL and also characterized by axonal losses, lack of myelin deposition due to oligodendrocytic losses, and astrogliosis. Congenital CMV infection and PVL affect the cerebral white matter in the same developmental period when immature oligodendrocytes are particularly vulnerable.

Introduction

White matter signal abnormalities on magnetic resonance (MR) imaging (MRI) may have different pathologic substrates. In a study of different leukodystrophies, proton MR spectroscopy, magnetization-transfer (MT) imaging (MTI) and diffusion-tensor imaging (DTI) have proven to be suitable techniques to discriminate between demyelination, hypomyelination, myelin vacuolation, white matter rarefaction and cystic degeneration (22). Static leukoencephalopathies were not included in that study.

Periventricular leukomalacia (PVL) is the most common brain injury in premature neonates and the leading known cause of cerebral palsy. PVL is caused by hypoxic-ischemic damage of the periventricular white matter. Characteristic MR imaging findings in children with PVL, after the first year of life, are a periventricular rim of hyperintense signal on T2-weighted images, volume loss of the periventricular white matter, and irregular ventricular contours (3). The abnormalities are static. The neuropathologic substrate of the periventricular white matter lesions in PVL is well documented and includes diffuse white matter gliosis, microglial activation, axonal loss and lack of myelin (5). The immature developing oligodendrocytes in the white matter are selectively vulnerable to hypoxia-ischemia resulting in hypomyelination (5).

Congenital cytomegalovirus (CMV) infection of the brain may also lead to damage of the cerebral white matter. Congenital CMV infection is the most common intrauterine infection in the western world, occurring in about 1% of all live births. Distinct MR imaging findings in children with a congenital CMV infection include multifocal lesions, predominantly involving the deep parietal white matter, relatively sparing the arcuate fibers and the white matter adjacent to the ventricles (13, 20, 23). Gyral abnormalities are present in some of the patients. The white matter abnormalities are static. In contrast to PVL, the nature of the white matter pathology is not clear; there is no literature on the subject.

To increase our insight into the nature of the white matter pathology related to the MR imaging findings in congenital CMV infection we applied MR imaging, spectroscopy and MTI and DTI in five patients with congenital CMV infection and six patients with PVL. Our hypothesis was that changes in quantitative MR parameters and MR spectroscopy concentrations in the static white matter lesions of PVL and CMV patients might be similar.

Materials and Methods

Patients and control subjects

This study was performed with informed consent of the patients, control subjects and parents, and with approval of the institutional ethics review body.

In a prospective study, we included five patients with a documented congenital CMV infection (age range 1.3 - 4.6 years; mean 2.9 years); they all fulfilled the MRI criteria for congenital CMV infection (23) and .in all patients a polymerase chain reaction (PCR)

technique revealed CMV DNA in blood spots, obtained in the neonatal period for screening purposes and stored on filter paper (Guthrie cards). We included six patients with PVL (age range 1.6 – 5.0 years; mean 2.8 years). The patients had a history of prematurity and hypoxia-ischemia in the neonatal period. The diagnosis of PVL was made when MRI revealed enlarged ventricles with an irregular outline, reduced white matter volume and abnormally high signal intensity of the periventricular white matter on T2-weighted images. They did not fulfill the MRI criteria for congenital CMV infection (23).

Quantitative MR imaging and spectroscopy results of the patients were compared with those of control subjects. Sixteen pediatric control subjects (age range 1.6 – 5.6 years; mean 3.1 years) were included in the study on the basis of their normal MRI.

MR imaging and spectroscopy

All MR studies were performed on the same 1.5 T MR scanner (Siemens Vision, Erlangen, Germany). The imaging protocol included sagittal T1-weighted images using a three-dimensional (3D) magnetization-prepared-rapid-acquisition-gradient-echo (MPRAGE) sequence (repetition time [TR] 15 ms, echo time [TE] 4 ms, 1 excitation), transverse T2-weighted spin echo images (TR 3000 ms, TE 22, 60 and 120 ms, 1 excitation), coronal or transverse FLAIR images (TR 9000 ms, TE 105 ms, inversion time [TI] 2200 ms, 1 excitation) and transverse diffusion weighted images using an echo-planar-imaging (EPI) sequence with b-values of 0, 500 and 1000 s/mm² (20 slices of 5 mm were acquired, with a 128x128 matrix, using TR 5100 ms, TE 137 ms). Automatically generated ADC maps were obtained.

DTI was performed in three CMV patients, in four PVL patients and in twelve control subjects with a multi-slice EPI-sequence according to the method described by Jones et al. (8) using a reference b=0 s/mm² and 8 non-collinear gradient vectors with b=1044 s/mm². In transverse orientation, 16 slices of 5 mm were acquired, with a 128 x 128 matrix, using TR 3600 ms and TE 123 ms. The DTI analysis included a correction of eddy-current induced distortion, and calculation of eigen-values of the diffusion tensor, ADC map and fractional anisotropy (FA) map (7).

MTI was performed in four CMV patients, in all PVL patients and in 10 control subjects with a 3D fast-low-angle-shot (FLASH) sequence. Two sets of images were obtained, one with (M_S) and one without (M_0) MT saturation pulse (7.68 ms Gaussian RF pulse, 1500 Hz off-resonance), using TR 23 ms, TE 4 ms, flip angle 20°, and a 3D-slab consisting of 54 transverse slices of 3 mm. MTR maps were created according to MTR=1-M_S/M_0.

In all PVL and CMV patients and in 10 control subjects MR spectroscopy was performed using a short-echo time stimulated echo acquisition mode (STEAM) sequence (TR/TE/mixing-time 6000/20/10 ms, 64 accumulations), in a single volume of interest (VOI) of 4-6 ml. In the control subjects the VOI was placed within the parietal white matter. In patients the VOI was selected in the center of the white matter lesion. Metabolite concentrations were calculated using LCModel and expressed as mmol/L. Concentrations

were determined for total creatine (tCr; creatine and phosphocreatine), total N-acetylaspartate (tNAA; N-acetylaspartate and N-acetylaspartylglutamate), choline-containing compounds (Cho), myo-inositol (mIns) and lactate (Lac) (16, 17).
Regions of interest (ROIs) corresponding to the MR spectroscopy VOIs were transferred to the equivalent ADC, FA or MTR maps. Mean values ± standard deviations (SDs) were determined for both patientgroups and controls.

Two sided unpaired t-test was performed to compare values between CMV patients and PVL patients and between both patient groups and control subjects. The significance-level was $p<0.05$.

Results

MRIs in all CMV patients showed more or less symmetric multifocal lesions in the deep parietal white matter, with sparing of the subcortical and the immediately periventricular white matter (Figure 1A, B).

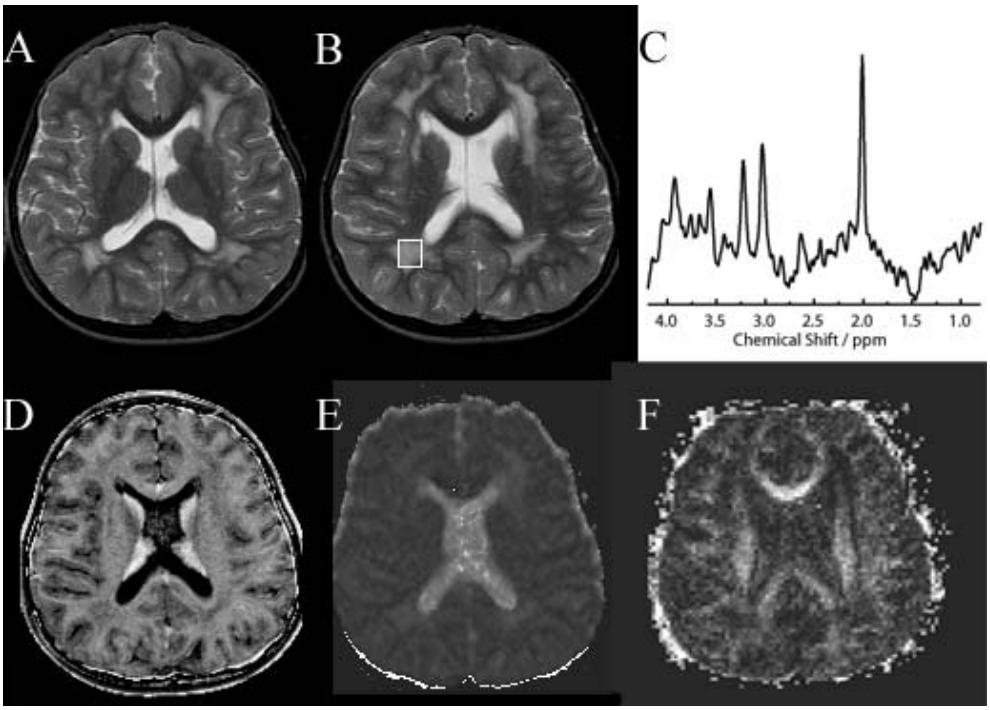

Fig. 1
(a, b). Transverse T2-weighted images (3000 / 120, 1 excitation) in a 4-year-old congenital CMV patient (patient 5) show symmetric multifocal lesions in the deep parietal white matter. (b) VOI localization for MR spectroscopy (STEAM TR/TE/TM = 6000 / 20 / 10 ms, 64 accumul-ations) on the transverse T2-weighted image with the corresponding spectrum (c) and equivalent transverse MTR (d) ADC (e), and FA (f) maps show increased values of ADC, decreased values of FA and MTR, and a decrease in tNAA (at 2.02 ppm) and Cho (at 3.20 ppm) concentrations.

In three of our patients (patients 2, 3 and 4) with congenital CMV infection some mild gyral abnormalities, with sometimes MR imging findings suggestive of a polymicrogyria, were present. No intracranial calcifications were seen. MRI studies in the PVL patients showed bilateral signal abnormalities in the cerebral white matter involving the parieto-occipital periventricular white matter. In all patients volume loss of the white matter with secondary dilatation of the lateral ventricles was observed. The ventricular border was irregular (Figure 2A, B).

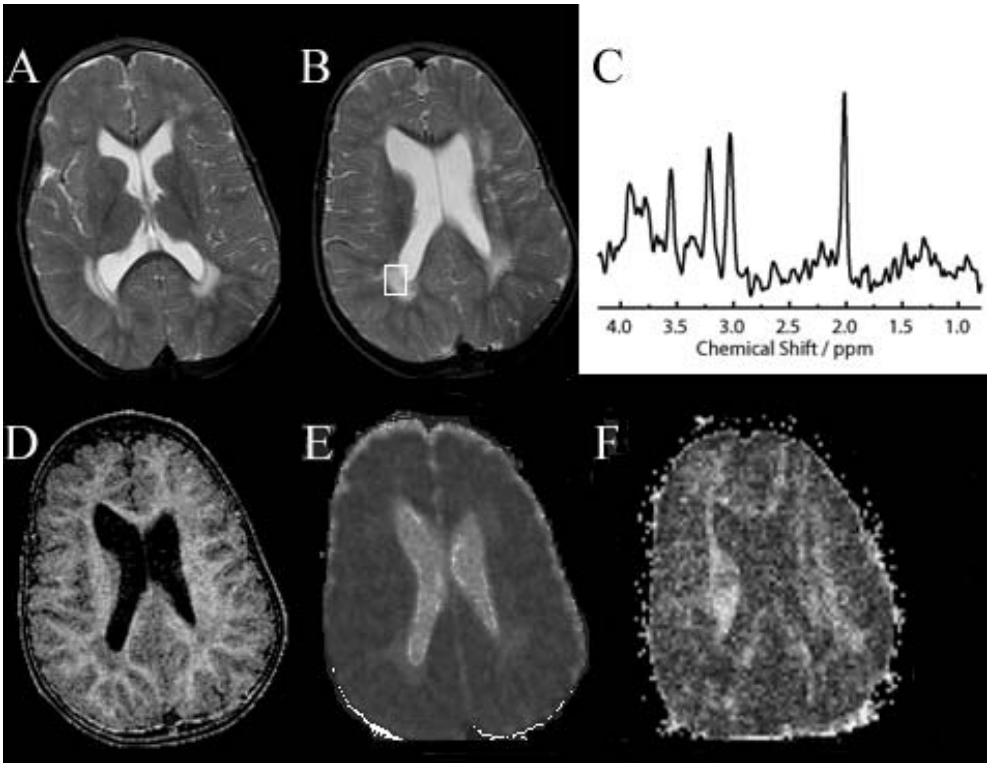

Fig. 2
(a, b). Transverse T2-weighted images (3000 / 120, 1 excitation) in a 3-year-old PVL patient (patient 6) show bilateral signal abnormalities in the cerebral white matter involving the parieto-occipital periventricular white matter with dilatation of the lateral ventricles and irregular ventricular border. (b) VOI localization for MR spectroscopy (STEAM TR/TE/TM = 6000 / 20 / 10 ms, 64 accumulations) on the transverse T2-weighted image with the corresponding spectrum (c) and equivalent transverse MTR (d) ADC (e), and FA (f) maps show increased values of ADC, decreased values of FA and MTR, and a decrease in tNAA (at 2.02 ppm) and Cho (at 3.20 ppm) concentrations.

Illustrations of proton MR spectra, MTR, ADC and FA maps that were obtained in the white matter lesions of CMV and PVL patients are shown in Figures 1C-F and 2C-F. The quantified metabolite concentrations and the results of DTI (or the ADC value from DWI, when DTI was not available) and MTI examinations are shown in Table 1.

Table 1: MTR (%), ADC (10^{-3} mm^2/s) and FA values and metabolite concentrations (mmol/L) in white matter regions of CMV and PVL patients and age matched controls

Patient	Gender	Age	MTR	ADC	FA	tCre	tNAA	Cho	Ins
CMV 1	F	2y5m	28.1	1.15	0.20	4.3	5.3	1.5	3.9
CMV 2	F	4y8m		1.24		3.7	6.0	1.0	4.1
CMV 3	M	1y4m	25.4	1.45	0.17	3.7	6.1	1.4	3.2
CMV 4	F	1y11m	27.0	1.35		4.8	6.3	1.7	3.6
CMV 5	M	4y1m	27.0	1.28	0.19	4.9	6.1	1.2	3.3
PVL1	F	1y11m	23.9	1.63	0.16	4.3	4.7	1.0	4.5
PVL2	F	1y7m	28.4	1.20	0.30	4.8	6.4	1.6	3.5
PVL3	M	2y1m	28.9	1.25		4.2	6.4	1.5	3.3
PVL4	F	3y2m	27.7	1.48		3.7	5.1	0.9	2.7
PVL5	M	5y	27.3	1.40	0.21	4.8	5.8	1.3	4.6
PVL6	F	3y4m	26.7	1.45	0.20	4.7	4.3	1.5	3.5
CMV		Mean	26.9	1.29	0.19	4.3	6.0	1.4	3.6
		SD	1.1	0.11	0.02	0.6	0.4	0.3	0.4
PVL		Mean	27.2	1.40	0.22	4.4	5.5	1.3	3.7
		SD	1.8	0.16	0.06	0.4	0.9	0.3	0.7
Controls		Mean	31.9	0.95	0.32	4.4	7.0	1.7	3.5
		SD	1.6	0.04	0.03	0.3	0.4	0.2	0.4

Note- Lactate was not detected

In the white matter lesions of both CMV and PVL patients, similar statistically significant changes in the quantitative MR parameters were observed as compared to controls, with increased values of ADC, and decreased values of both FA and MTR. Furthermore, changes in metabolite concentrations as determined by MR spectroscopy were similar. In both patient groups a statistically significant, marked reduction in the concentrations of tNAA and Cho was observed as compared to the control subjects. The concentration of mIns was slightly elevated in both patient groups as compared to the controls, but this change did not

reach the level of significance. tCr concentrations in patients were similar to that of controls. Lactate was not detected in any of the spectra.
No statistically significant differences between the two patient groups were found for MTR, ADC and FA values, nor any of the metabolite concentrations.

Discussion

By comparing the changes in quantitative MR parameters and MR spectroscopy metabolite concentrations in the white matter lesions between CMV and PVL patients we intended to determine whether the nature of the white matter pathology in congenital CMV infection could be similar to the known pathology of PVL. Diffusion parameters, MTR and MR spectroscopy concentrations were studied in cerebral white matter lesions in five patients with a congenital CMV infection, six patients with PVL, and in normal individuals. In both patient groups ADC values were highly increased, FA and MTR values were reduced, and tNAA and Cho concentrations were reduced as compared to healthy individuals; mIns concentrations were slightly increased. No differences were found between the two patient groups.

Congenital CMV infection is a major public health concern. CMV infection is the leading cause of congenital infections in developed countries, occurring in approximately 1% of all live births (13, 20, 23). Only 10% of infected children demonstrate symptoms at birth with signs including jaundice, hepatosplenomegaly, microcephaly and thrombocytopenia; while the remainder is asymptomatic. Both symptomatic and asymptomatic CMV infection may cause a wide spectrum of neurological abnormalities. On the severe end of the spectrum are severe motor handicap and mental retardation; on the mild end are learning, behavioural and motor coordination problems (13, 20, 23). The severity of neurological abnormalities may be related to the stage of central nervous system development, at which congenital infection occurred (1, 12). Early infections affect neuronal growth and migration; their pathologic features in the injured brain are well documented and include pachygyria, polymicrogyria, cerebellar hypoplasia and calcifications (12). Later-onset infections supposedly leave neuronal migration and organization unaffected and mainly lead to cerebral white matter lesions. The pathology of these white matter lesions has, as far as we know, not been studied, and the pathogenetic mechanisms by which CMV may affect the white matter are, therefore, unclear.

In general, several potential pathogenetic mechanisms by which CMV may injure the central nervous system have been suggested. These include CMV acting as a teratogen disrupting normal cellular differentiation, and CMV inducing apoptosis. Studies in animal models and in cell cultures revealed that a wide variety of cells in the central nervous system can be infected: astrocytes, oligodendrocytes, neurons and brain endothelial cells are all permissive for CMV infection (11, 12, 15). CMV-infected cells are located predominantly in the ventricular and subventricular zones (12), and the virus may therefore disrupt the health and function of neural stem cells in these regions in the immature brain. Infection of the developing mouse brain was shown to disturb neuronal migration and induce loss of

neuronal cells due to either inhibition of the cell cycle or increased cell death by apoptosis (15). CMV inhibits the induced differentiation of neural precursor cells into neurons, and can have the same inhibitory effect with respect to the differentiation into other cells, such as macrophages and dendritic cells (12). It is likely that the virus may disrupt the maturation of oligodendrocytes as well. In addition, alterations in the microenvironment of the developing brain due to cytokines generated by resident glial cells and infiltrating immune cells may induce apoptosis of cells in the fetal brain.

White matter injury known as PVL, mainly occurring in premature infants, is due to the ischemic damage to the periventricular white matter, the watershed zone in the developing foetus. The pathology of the injured white matter is characterized by diffuse lack of myelin, gliosis and axonal losses. The number of oligodendrocytes is decreased and they often lack processes (5). Immature oligodendrocytes are in a phase of active development during weeks 24-40 of gestation. During the peak period of PVL these cells predominate in cerebral white matter and start to differentiate into mature oligodendrocytes. This renders them especially vulnerable to injurious insults, such as ischemia and inflammation, leading to cell loss or loss of processes with intact soma. The result would be a lack of mature oligodendroglia and a consequential impairment of myelination (3, 9, 18, 24). In addition, injury to the immature oligodendrocytes may also lead to failure of axonal development and ultimately axonal degeneration (24).

Congenital CMV infection and PVL affect the cerebral white matter in the same developmental period and both lead to static lesions. In this study similar changes were found in quantitative MR parameters and MR spectroscopy concentrations in the white matter lesions of PVL and CMV patients.

Within the white matter lesions, ADC, a measure for isotropic water diffusivity, was increased, and FA, a measure for the degree of diffusion anisotropy, was decreased. This indicates enhanced mobility of water molecules in all directions as a result of damage to the tissue matrix. These changes reflect loss of tissue in the injured white matter, with a reduction in the number of axons and myelin sheaths, and increased interstitial spaces (4, 6, 14).

The MTR in the white matter lesions was decreased indicating a reduced capacity of the macromolecule-bound protons in brain tissue to exchange magnetization with the surrounding protons in free water. Similar to the diffusion parameters, this reflects damage to myelin and axonal membranes in the white matter with losses of oligodendrocytes, myelin sheaths and axons (21).

Quantitative localized proton MR spectroscopy of white matter lesions revealed decreases of tNAA, consistent with axonal losses. The low Cho concentration is consistent with hypomyelination of the affected white matter (2, 7, 10, 19).

The concentration of tCre was normal, despite the axonal loss. Together with the relatively high concentration of mIns this reflects astrogliosis of the injured white matter (2, 7, 10, 19).

Our diffusion parameters and MR spectroscopy findings are in agreement with previous reports on PVL (1, 11, 12). The observed changes in MR parameters in the white

matter lesions in the PVL are in line with the known underlying pathologic changes, including axonal loss, lack of myelin deposition in the damaged white matter and white matter gliosis (5).

Because of the nature of the changes in MR parameters and because of the similarity between the two patient groups, the results of our study suggest that the pathologic substrate of the white matter lesions in congenital CMV infections is also characterized by axonal losses, lack of myelin deposition due to oligodendrocytic losses, and astrogliosis. One of the most important factors in the development of white matter abnormalities in both congenital CMV infection and PVL is probably found in damage and loss of oligodendrocytes within the developing white matter.

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