Chapter 8

No structural cerebral differences between children with a history of bacterial meningitis and healthy siblings

Rogier C.J. de Jonge
Joost F. Swart
Irene Koomen
Serge A.R.B. Rombouts
Reinoud J.B.J. Gemke
Frederik Barkhof
A. Marceline van Furth

Both authors Rogier de Jonge and Joost Swart contributed equally to this manuscript

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ABSTRACT

Aim:
Bacterial meningitis is a serious infection of the central nervous system. About one-third of affected children develops academic and/or behavioral limitations after recovery from bacterial meningitis. The aim of our study was to search for structural differences in the brain, with a special focus on the hippocampus, between childhood survivors of bacterial meningitis with and without academic and/or behavioral limitations and healthy siblings.

Patients and Methods:
A selection of an existing cohort, compiled in an earlier performed retrospective study was used in this case-control study. Magnetic Resonance Imaging scans of the brain were performed in 43 post-meningitis children, of whom 18 had learning and/or behavioral limitations, 25 had no problems, and 18 siblings with no history of bacterial meningitis. With Voxel-based morphometry the brain was investigated for local structural changes and hippocampal volume and lateral ventricle width were measured on both sides.

Results:
There were no structural differences between the groups in any area of the brain. There were no significant differences between the three groups in hippocampal volume or lateral ventricle width. The group with limitations had three children with a right hippocampal volume smaller than 2 SD below the mean.

Conclusion:
Despite hippocampus lesions that were found in experimental bacterial meningitis studies, we found no persistent anatomical differences of the brain or hippocampus related to bacterial meningitis in children, nor to the academic and/or behavioral limitations seen after bacterial meningitis.
INTRODUCTION

Bacterial meningitis (BM) is a serious infection of the central nervous system (CNS) that affects about 5 per 100,000 children in the Netherlands each year. It is mainly caused by Neisseria meningitidis and Streptococcus pneumoniae. Severe sequelae, such as hearing loss, motor problems, seizures and mental retardation occur in 15% of the children who survive BM. More subtle adverse outcomes such as cognitive, academic and behavioral limitations are present in more than 20%.

For the Dutch population, Koomen and co-writers reported an incidence of academic and/or behavioral limitations of 32% among children who survived bacterial meningitis. In this study, 182 children with a history of non-Haemophilus influenzae type B meningitis underwent a neuropsychological evaluation. In general, children with academic and/or behavioral limitations following BM performed poorly on measures of cognitive functioning, speed and motor steadiness.

BM can lead to severe damage of cerebral tissue which is mediated by a complex system of inflammatory pathways such as pro- and anti-inflammatory cytokines, reactive oxygen intermediates, and by ischemia which is caused by vasculitis and cerebral edema. From experimental meningitis data it is known that areas of necrosis are mainly present in the cortex and neuronal loss due to apoptosis is primarily seen in the dentate gyrus of the hippocampus. In humans, only a few studies are available which also demonstrate structural changes to the hippocampus due to BM. Apoptosis of hippocampal cells was demonstrated in 26 human autopsy cases that died of BM, while in 10 age-matched controls, no damage was found. Also bilateral atrophy of the hippocampus was found in patients with temporal epilepsy and a history of encephalitis or meningitis.

The hippocampus plays an essential role in learning and memory. Hippocampal damage as a result of hypoxia, surgery or chemical irritation (in experimental studies) has been highly associated with learning disorders in both human and animal studies. This was also confirmed in experimental meningitis studies, where a bad performance in the Morris Water Maze by rats with pneumococcal meningitis correlated with a profound damage in the hippocampus.

Although literature supports the idea that hippocampal lesions mainly contribute to impaired spatial functioning, a recent study demonstrates that the human hippocampus participates in both spatial and non-spatial configural learning.

Learning and memory are complex processes, involving more cerebral systems than only the hippocampi. A recent study in humans who survived BM showed significant disturbances in short-term and working memory, which correlated with brain atrophy and the number of lesions found in the white matter, suggesting that more diffuse damage of the brain after BM is responsible for limitations later in life.

Although quite a lot data from experimental research is available, no research has been done in humans regarding hippocampal damage in BM survivors and the correlation with academic
and/or behavioral limitations. No cerebral imaging studies in children after BM have been done at all. The main objective of this study was to investigate whether structural differences in the brain, especially the hippocampus, exist between childhood survivors of BM and healthy children. The second goal was to study the correlation between possible anatomical changes of the brain and BM related academic and/or behavioral limitations.

METHODS

Subjects
The studies of Koomen et al. formed the basis of our study in which we performed MR imaging of post-meningitis children with and without academic and/or behavioral limitations. The rationale and design of these studies has been extensively described. The cohort existed of 182 children (mean age 10 years; range 5-14 years) with a history of BM caused by *N. meningitidis*, *S. pneumoniae*, *Streptococcus agalactiae*, *Escherichiae coli* or *Listeria monocytogenes*. The children suffered from BM between January 1990 and December 1995 (median age 2.9 years, range 0-6.9 years). All patients with a complex onset of BM (defined as: meningitis secondary to immunodeficiency states, central nervous system surgery, cranial trauma or cerebrospinal fluid shunt infection, or relapsing meningitis), pre-existing cognitive or behavioral problems or severe handicaps were excluded. A nested case-control design was used for neuropsychological evaluation. Academic limitations were found using an Academic Achievement Test (AAT), assessing written arithmetic, reading aloud and reproducing stories, writing to dictation, and copying sentences using tasks taken from standard educational packages. Behavioral limitations were identified with the “Child Behavior Checklist”. In 84 of the 182 children academic and/or behavioral limitations were found. A prediction rule based on nine risk factors was formulated to identify post-meningitis children at high risk of developing academic and/or behavioral limitations. A sample of the original cohort was selected for this case-control study. We excluded children younger than 12 years at the time of the study and randomly selected and approached 43 children with proven limitations and 42 children without problems. The minimum age of 12 was chosen to make sure the children were able to undergo scanning without sedation. Nineteen of the 43 children with limitations were included, the other 24 children were excluded. From the 42 children without limitations 26 were included, the other 16 were excluded. Reasons for exclusion were: the child had fixed dental braces, moved to an unknown address or did not want to participate. In both the groups of children with and without limitations one MRI scan could not be completed because of anxiety of the child. Furthermore, 18 healthy siblings were included; eight were related to a child with limitations and 10 to a child without problems. Figure 1 presents the patient flow chart.

All studied children with a history of BM were between 12 and 18 years old at the time of their MRI scan, and the scans were performed 8-14 years after the disease. The experiments
were undertaken with the understanding and written consent of each subject and parents or responsible guardian, with the approval of the appropriate local ethics committee. The selected population was compared with the original cohort of 182 children regarding the basic patient characteristics: “gender,” “age at time of neuropsychological evaluation” and “IQ at time of neuropsychological evaluation” to investigate if selection bias in these variables were possible confounders.

**Figure 1**: Patient flow chart

Magnetic resonance image acquisition

Whole-brain MRI scans of the 61 children were obtained. Imaging was performed on a 1.5 T Magnetom Vision system (Siemens AG, Erlangen, Germany) scanner using a standard circularly polarized head coil, with foam padding to restrict head motion. A localizer scan was first performed for positioning of the image planes, followed by an automated shim procedure to improve magnetic field homogeneity. Scans were obtained as whole-brain 3D heavily T1-weighted MPRAGE (magnetization prepared rapid acquisition gradient echo) volumes and were acquired in the coronal plane (inversion time 300 ms, repetition time 15 ms; echo time 7 ms; flip angle 8°; matrix 256x256; 160 slices; voxel size 1x1x1.5 mm).
**MRI data analysis**

Three procedures were used to analyze the MRI data. The co-workers that performed these procedures were not informed about the history of the volunteer.

(1): **Voxel-based morphometry (VBM)** was performed. This is a fully automated, sensitive, standardized, whole-brain technique in which images of each subject are normalized by transforming all subjects’ data to the same standard stereotactic space \(^{24}\). Analysis was performed using statistical parametric mapping software (SPM2; Wellcome Department of Cognitive Neurology, Institute of Neurology, London) with the VBM tool Jena script in MATLAB version 6 (Mathworks, Natick, MA, USA). We used optimized VBM with a custom template and priors because this method improves the plausibility of intergroup comparisons, presumably because of improved segmentation and spatial normalization \(^{25}\). The processing steps outlined by this protocol are: (1) the creation of a customized anatomical T1-weighted template and prior probability images: this was done by normalizing the brain images of each participant to the default SPM T1-weighted template. These normalized images of all participants were averaged to create the custom template. It should be noted that the custom template was created using MR images of all participants. Each participant’s normalized images were also segmented. The segmentation step also incorporates an image intensity non-uniformity correction to address image intensity variations caused by different positions of cranial structures within the MRI head coil \(^{24}\). Averaging these segmented images created the customized prior probability images. Details on the evaluation of the VBM segmentation technique, including evaluation of the non-uniformity correction and stability with respect to misregistration with the a priori images are available in Ashburner and Friston \(^{24}\). Finally, both the custom template and the customized prior probability images were spatially smoothed using a Gaussian kernel of 8 mm to obtain the final template and probability images used for further analysis; (2) the normalization of the structural brain images in each group using these customized templates and prior probability images, segmenting and cleaning the original T1-weighted images. Statistical analysis used the general linear model and is based on the random Gaussian field theory \(^{26}\). Grey matter images were spatially smoothed using a Gaussian kernel of 8 mm. We performed analyses on the smoothed grey matter images only. Differences in grey matter density (GMD) between the groups were examined using t-tests, and, as described before, we compared the total group of post-meningitis children with or without limitations with the control group of siblings, the group of post-meningitis children with limitations to the group without limitations, and the post-meningitis children with limitations to the control group.

Results were thresholded at \(p\)-value < 0.05 corrected for multiple comparisons at the cluster level. Additionally, we applied a less stringent threshold (\(p\)-value < 0.01, uncorrected), to be more sensitive to possible subthreshold differences.
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(2): **Absolute volumes of the hippocampus** were measured bilaterally using the method of recognition points as described by Jack et al. 27, 28. Volumetry of both hippocampi was performed because of possible unilateral atrophy. Volumetry was performed according to standard operating procedures at our institute in which voxels within a region-of-interest (ROI) were counted. The rater involved in this manual method of volumetry was trained to have an inter-observer error less than 5% according to our own gold standard. Absolute volumes were corrected for total brain volume (TBV), the sum of grey and white matter, without cerebrospinal fluid. This TBV was obtained from the Voxel-based morphometry procedure, as be discussed earlier in this article.

(3): **The radial width of the temporal horn of the lateral ventricles** was measured bilaterally. This technique is validated as a sensitive marker in Alzheimer disease, in which hippocampal volume loss is one of the most prominent neuro-radiological findings 29.

For these two procedures, differences between hippocampal volumes and differences in radial width between the groups were tested by Mann-Whitney-U-test for non-parametric testing, using SPSS statistical software edition 13.0 (SPSS, Chicago, US). We compared the total group of post-meningitis children with or without limitations to the control group of siblings, the group of post-meningitis children with limitations to the group without limitations, and the post-meningitis children with limitations to the control group.

**RESULTS**

In Table 1 the basic patient-characteristics of the study-group children with a history of BM versus the original cohort are compared. There were no significant differences in gender and the results of the intelligence-test. There was a significant difference between the groups in the mean age at time of neuropsychological evaluation.

**Voxel-based morphometry**

The voxel-wise, whole brain analyses did not reveal any group difference for any test, even when applying the uncorrected threshold (of $p$-value $<$0.01).

**Volumetry**

The median absolute volumes of the hippocampi on both sides, median TBV and the hippocampal volumes corrected for TBV were calculated for the three groups separately and for the total group of post-meningitis children (Table 2). Only the absolute volume of the left hippocampus appeared to be slightly decreased in children with academic and/or behavioral limitations after BM, compared with children without a history of BM, however, this was not significant ($p$-value 0.1) (Table 2).
Radial width of the lateral horns

The median width of both lateral horns was calculated for the three groups separately and for the total group of post-meningitis children (Table 2). No differences were found between the radial widths of the lateral horns on the left or right sides.

Because of small sample sizes and the lack of significant differences we looked at individual analyses of the children with a history of BM to see if there was a striking amount of patients

Table 1: Comparison of patient-characteristics study-group vs. original cohort

<table>
<thead>
<tr>
<th></th>
<th>post-meningitis children in study-group (n=43)</th>
<th>all post-meningitis children of original cohort (n=182)</th>
<th>t-test, p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (% male)</td>
<td>56</td>
<td>64</td>
<td>0.38</td>
</tr>
<tr>
<td>Age at time of neuropsychological evaluation of the original cohort (yrs.)</td>
<td>10.6 (1.5)</td>
<td>9.7 (2.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>IQ at time of neuropsychological evaluation of the original cohort</td>
<td>99.5 (16.3)</td>
<td>101.3 (13.8)</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Table 2: Results of hippocampal and brain volumetry and measurement of radial width of ventricular lateral horns (all groups)

<table>
<thead>
<tr>
<th></th>
<th>Total group post-meningitis children (n=43) (median, range)</th>
<th>Post-meningitis children with academic and/or behavioral limitations (n=18) (median, range)</th>
<th>Post-meningitis children without academic and/or behavioral limitations (n=25) (median, range)</th>
<th>Control group: Siblings without meningitis (n=18) (median, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>14.3 (12.3-17.9)</td>
<td>13.9 (12.8-17.9) †‡</td>
<td>14.9 (12.3-17.9) †</td>
<td>15.0 (12.1-17.9) †</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>53.5</td>
<td>61.1</td>
<td>48.0</td>
<td>55.5</td>
</tr>
<tr>
<td>Hippocampal volume left (ml)</td>
<td>2.5 (1.9-3.1)</td>
<td>2.3 (1.9-3.1) †</td>
<td>2.6 (2.0-3.0)</td>
<td>2.7 (2.1-3.1) †</td>
</tr>
<tr>
<td>Hippocampal volume right (ml)</td>
<td>2.6 (2.0-3.6)</td>
<td>2.8 (2.0-3.6)</td>
<td>2.6 (2.2-3.2)</td>
<td>2.7 (2.1-3.1)</td>
</tr>
<tr>
<td>Total brain volume (TBV) (ml)</td>
<td>1186.1 (998.5-1411.0)</td>
<td>1155.7 (998.5-1313.2)</td>
<td>1191.2 (1001.6-1411.0)</td>
<td>1181.4 (958.9-1408.4)</td>
</tr>
<tr>
<td>Hippocampal volume left corrected for TBV (%)</td>
<td>0.22 (0.16-0.27)</td>
<td>0.21 (0.16-0.27)</td>
<td>0.22 (0.18-0.26)</td>
<td>0.22 (0.18-0.27)</td>
</tr>
<tr>
<td>Hippocampal volume right corrected for TBV (%)</td>
<td>0.23 (0.17-0.28)</td>
<td>0.22 (0.17-0.28)</td>
<td>0.23 (0.17-0.28)</td>
<td>0.23 (0.18-0.28)</td>
</tr>
<tr>
<td>Radial width of the lateral ventricle left (mm)</td>
<td>2.8 (1.8-5.4)</td>
<td>2.9 (1.8-5.4)</td>
<td>2.8 (1.9-4.5)</td>
<td>2.9 (2.0-5.5)</td>
</tr>
<tr>
<td>Radial width of the lateral ventricle right (mm)</td>
<td>3.4 (1.7-5.8)</td>
<td>3.6 (1.7-5.8)</td>
<td>3.2 (1.9-4.9)</td>
<td>3.8 (1.5-4.9)</td>
</tr>
</tbody>
</table>

* Mann-Whitney U test, p-value 0.1, comparison post-meningitis group with limitations vs. post-meningitis group without limitations
† Mann-Whitney U test, p-value 0.1, comparison post-meningitis group with limitations vs. control group
with hippocampal volumes, TBV and radial width below -2 standard deviations of mean values of the control group (Table 3). The group with limitations presented one child with left and three children with right hippocampal volume smaller than two standard. Corrected for TBV both sides showed one child with this deviation. In the post-BM group without limitations one child had a small hippocampus, corrected for TBV there was one child with a deviating right hippocampus. In the control group one child with a right hippocampal volume and one with a TBV smaller than two standard deviations below the mean was found.

**DISCUSSION**

Many studies provide evidence that the brain, and especially the cortex and the hippocampus is damaged in humans after BM and that this damage may be correlated with academic and/or behavioral limitations. In this study we could not confirm this data. No volume loss or structural changes of the brain were found, nor did we find differences in volume between the hippocampi of healthy children and childhood survivors of BM, not even in survivors with academic and/or behavioral limitations.

To appreciate the results of this study, the following points need to be discussed. First of all, every imaging technique has its limitations and graphical resolution might not be optimal. However, MRI volumetry and VBM are very sensitive tools for finding small differences in brain tissue. Hence, it is very unlikely that any clinically relevant (hippocampal) atrophy was missed.

<table>
<thead>
<tr>
<th>Table 3: Number of individual children with noticeable deviations from controls (below -2 SD of the mean value of the control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group:</strong></td>
</tr>
</tbody>
</table>
| Siblings without meningitis (n=18) (-2 SD - mean) | <-2 SD of controls (individual values) | <-2 SD of controls (individual values) | <-

| Hippocampal volume left (ml) | 2.05 - 2.66 | 1 (1.94) | 1 (1.98) | 0 |
| Hippocampal volume right (ml) | 2.17 - 2.76 | 3 (1.97; 2.10; 2.16) | 0 | 1 (2.12) |
| Total brain volume (TBV) (ml) | 974 - 1193 | 0 | 0 | 1 (958) |
| Hippocampal volume left corrected for TBV (%) | 0.17 - 0.22 | 1 (0.16) | 0 | 0 |
| Hippocampal volume right corrected for TBV (%) | 0.18 - 0.23 | 1 (0.17) | 1 (0.17) | 0 |
| Radial width of the lateral ventricle left (mm) | 1.3 - 3.2 | 0 | 0 | 0 |
| Radial width of the lateral ventricle right (mm) | 1.4 - 3.4 | 0 | 0 | 0 |
by the tests we used. In our opinion, the lack of structural differences between the groups reflects the absence of (hippocampal) atrophy in the brain after BM. Nevertheless, although VBM is a very sensitive tool, Bernasconi et al. described T2 relaxometry as a superior technique to find damage in normal volume hippocampi. This might have been a good alternative for focusing on hippocampal damage.

Secondly, although strong evidence is available in the literature that academic and/or behavioral limitations are related to hippocampal damage, the hippocampus is not the only cerebral region responsible for cognitive and personality functions. It is a part of a complex system in which the cortex also plays an important role. We realize that the tests used for the selection of children with limitations is not specific for hippocampal functions, but represents an overall idea of cognitive and behavioral impairment. However, our study did not find any structural changes in cortical or other regions of the brain, either.

Third, our study was performed with relatively small groups, and it is possible that small differences were not detected. The fact that differences in left hippocampal volumes between the control group and the post-BM group with problems were not significant could be due to small sample size. However, differences in hippocampal volumes corrected for TBV did not even show a trend towards a significant difference, so we believe that it is unlikely that any clinically relevant differences were missed. Moreover, because of the small sample sizes we analyzed the patients individually (Table 3), to see if there was a noticeable number of children with deviations from the control group. This analysis showed no obvious differences between the three groups, except that three children in the post-BM group with limitations had a right hippocampal volume smaller than 2 standard deviations below the mean.

Finally, this study has been based on a retrospective selected cohort from which two groups of children were selected and included. Hence, selection bias could be a problem. To evaluate this issue we compared gender, age, and intelligence between our study-group children after BM and the original cohort of patients. There were no differences in the distribution of gender and intelligence, making selection bias by subjects with fewer limitations not plausible. However, there was a difference in age, with significantly older children in our study group. This needs to be explained by our selection of children older than 12 years at the time of MRI. Using hippocampal volumes corrected for TBV, the influence of age on the results is believed to be neglectable.

In the light of our results it is an important question whether there could have been (measurable) damage of the brain just after the acute phase of the BM, but due to neurogenesis the cerebrum has been able to recover from anatomical changes. This hypothesis has been described by recent work done with animal models after meningitis or a hypoxic event. These studies strongly suggest that functional and structural recovery of the hippocampus is related to the ability of neurogenesis of the organism, and that younger animals have a more active neurogenesis. To look for damage in the early phase of BM, a MRI scan shortly after the acute illness needs to be done, however, not much research has yet been performed regard-
ing early cerebral MR imaging. One group that performed MRI in early BM only investigated a selected, very ill pediatric population with therapy-resistant BM. Serious complications of BM, like multiple infarction and empyemas were found, but no conclusions about hippocampal involvement were drawn. Schmidt et al. recently published a study in which MRI scans of the cerebrum and neuropsychological tests were performed in adults 1-12 years after BM or viral meningitis (VM). The causative bacterial pathogens were comparable with those in our population. Measurements of TBV and ventricular volume were done and they also scored the number of white matter lesions associated with BM. Patients after BM had significantly more memory and learning problems than controls and VM patients, and a correlation was found with signs of cerebral atrophy and white matter lesions. In our study, white matter lesions were identified so infrequently during the blinded review by an expert neuroradiologist, that a formal analysis of such lesions was dismissed as being extremely unlikely to reveal group differences. In adults who survived BM, cerebral atrophy was measurable even a significant time after BM, supporting our hypothesis that the stronger ability of the child’s brain to recover from neuronal damage might be an explanation for our results. Another explanation for the hippocampal damage reported in these studies, in contrast to our findings, might be the severity of the meningitis in comparison to our population; these studies regarded autopsy results in deceased adults, and patients with persistent epilepsy after meningitis. Again, it would be very interesting to perform MRI scans in children shortly after the acute phase of BM, and to compare these results and the outcome of our paper with MRI images of the adult brain after BM.

In conclusion, this study found no persistent anatomical differences of the brain or hippocampus related to BM in children, nor to the academic and/or behavioral limitations seen after BM. But we strongly believe that more research providing information about the damage of the hippocampus after BM in childhood is needed. In the near future we would like to perform MRI scans shortly after the acute phase of BM in children, and compare these results and the outcome of this paper with the adult brain after BM. Further, fMRI or PET-scanning might reveal functional damage caused by BM.
REFERENCES


