**In vivo Study of Experimental Pneumococcal Meningitis Using Magnetic Resonance Imaging**

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Abstract

The present study aimed to follow and describe the evolution disease pathophysiology in experimental pneumococcal meningitis by using \textit{in-vivo} Magnetic Resonance Imaging. Rats infected \textit{S. pneumoniae} (n=29) or saline (n=13) were randomized for imaging at 6, 12, 24, 30, 36, 42 or 48 hours after infection. T1W, T2W, quantitative diffusion, and post contrast T1W were acquired at 4.7T. Dynamic MRI was used to evaluate blood-brain-barrier (BBB) permeability and to obtain measures of cerebral- and muscle perfusion.

The present study enabled visualization and delineation of the significant BBB-breakdown and ventricle expansion associated with pneumococcal meningitis (P<0.002 and P<0.0001) whereas heterogeneous changes in cerebral ‘perfusion’ and brain water distribution was observed. Ventricle expansion was strongly associated to disease severity and developed concomitant to reduced cerebral vessel leakage suggestive of cortical compression. Areas of well –‘perfused’ muscle decreased with the progression of infection indicative of an increasing septicaemic component (P=0.05).
The development and progression of bacterial meningitis is associated with multiple pathophysiological changes in brain homeostasis. This has been investigated experimentally in studies employing a number of advanced methodologies able to determine the kinetics of infection and inflammation; loss of blood-brain-barrier and blood-labyrinth–barrier; damage to brain cortex, hippocampus and white matter; development of brain oedema; alterations in brain blood supply and loss of cerebral vascular autoregulation [1-6]. However, experimental investigations of the interaction and interplay between these pathophysiological measures have been limited, probably due to the complexity and incompatibility of the methodology applied. Recent bioluminescence studies illustrate the apparent limitation of a single method. The optical technique is able to visualize the dynamics of the progressing meningeal infection but is unable to provide additional information relating to disease induced physiological changes [7;8].

Magnetic Resonance Imaging (MRI) has been widely used for the study of experimental stroke [9] but has, to our knowledge, only been applied once for the study of meningitis in an experimental rat model [10], focusing on image analysis of meningeal enhancement and hydrocephalus. When compared to experimental stroke studies that aim to produce single lesions localised in predetermined anatomical sites, bacterial meningitis results in diffuse and unpredictable involvement of brain vasculature and parenchyma that is further complicated by systemic infection.

In clinical neuroinfections, including meningitis, MRI has primarily been used as a diagnostic tool to assess brain pathology, intracranial complications and to evaluate responsiveness to treatment [11;12], despite MRI’s capability to provide quantifiable in vivo data on BBB function, brain water distribution as well as indices of cerebral blood supply [9].
Consequently, the aim of the present study was to investigate whether MRI methodology could be used to acquire multi-parametric \textit{in-vivo} data in studies of experimental meningitis. MR measures of inflammation, vascular permeability, brain water and blood supply could be compared with standard histological, clinical and paraclinical data, potentially providing further insight into the evolution of the disease.
Materials and Methods

The experimental protocol was approved by the Danish Animal Inspectorate (Dyreforsoegstilsynet). Adult male Wistar rats (280-300 g) were used for the experiments. Normal day/night cycles and free access to food and water were provided.

Experimental study design

Data from 29 rats infected with pneumococci and 13 controls inoculated with saline are presented. Four infected and 2 control rats were randomized to MR examination at 6, 12, 24, 30, 36, 42 or 48 hours following inoculation. Immediately prior to MR investigation, each rat was assessed clinically and neurologically (see table 1). After imaging, blood and CSF samples were taken and brains prepared for histopathology (see below). Two infected rats assigned to MR imaging at 24 and 36 hours after infection and 1 control rat assigned to MR imaging at 48 hours died in the scanner. Infected rats were replaced from a separate group (incorporated due to the risk of sick rats dying from anesthesia, n=4) that was not assigned to a specific time point a priori.

Infection

A *Streptococcus pneumoniae* type 3 strain (68034, Statens Serum Institut (SSI), Copenhagen, Denmark) was used for the experiments. The infectious inoculum was diluted in cold saline to a final concentration of 2-5 x 10^5 CFU/ml, as confirmed by quantitative cultures. On the day of inoculation rats were anaesthetized with aqueous solution of Hypnorm®/Dormicum®/Atropine and injected intracisternally with 30 µl of the bacterial suspension or saline. Sampling of cerebrospinal fluid (CSF) and blood were obtained from each animal after MR investigation. White blood cell (WBC) counts in CSF were measured on an automatic cell counter (Swelab Autocounter AC 920, Swelab Instruments, Sweden) using 20 µl of CSF. Bacterial counts in CSF were determined by
plating 10-fold serial dilutions of 20 µl CSF. Fifty µl of undiluted blood and a 20-fold
dilution was also plated. CSF drawn from uninfected controls were plated undiluted.

Assessment of clinical disease, motor performance and disease severity (Table 1)
Clinical appearance and motor-function scores were determined prior to each scanning
session. The scoring method was based on previous reports and fitted to the present model
of disease [1;13;14], see table 1. Overlap between scores was evident since, for example,
ambulatory activity would be affected by clinical disease.

MR imaging
MR imaging were performed using a Varian SISCO 4.7 Tesla imaging and spectroscopy
system. Rats were positioned in a stereotactic device placed within a home-built
quadrature coil. The animals were kept warm using a blanket and circulating warm water.
All rats underwent T1W, T2W, quantitative diffusion, dynamic MRI (dMRI) and post
contrast T1W measurements whilst anaesthetized as described above. To enable direct
comparison of the imaging data, the same 12 contiguous coronal slices were acquired for
the T1W, T2W and diffusion measurements. The acquisitions using a b-value =0 provided
the T2W images. Three coronal MR images corresponding to the frontal, mid-frontal and
mid-brain were selected for dynamic MRI investigation, measurement of ventricular-brain
ratio and calculation of ADC.

ADC - Apparent diffusion coefficient mapping
Quantitative diffusion measurements (single in-plane direction, along the x-axis) were
performed before the administration of contrast agent (TE=80ms, TR=2000ms,
MA=128x128, FOV=40x40mm, NT=1 with b-values of 0, 185, 740, 1665 s/mm²).
Regions of interest were drawn, in cerebral neo-cortex and basal ganglia in both right and
left hemispheres, independently by two of the authors (HS and CTB) blinded to all other
data. A mean ADC for value was calculated in MATLAB©.
Dynamic MRI - Loss of blood-brain-barrier (BBB) integrity and clustering of contrast delivery (perfusion) to cerebral cortex and muscle (Fig. 1).

T1W images ((TE=11ms, TR=450ms, SL=1.8 mm, MA=128x256, FOV=40mm x40mm, NT=4, 12 contiguous slices) were acquired before and after bolus administration of contrast agent (GdDTPA, Magnevist, Schering AS) via a cannulated tail vein. The contrast agent was injected within 10 seconds, at a dose of 0.5 mmol/kg. Bolus passage was followed using a dMRI protocol where 100 sets of FLASH T1W images (3 slices) were obtained with 10 images acquired before and 90 images acquired after injection of contrast agent (TE=4ms, TR=11ms, FL=7º, SL=1, MA=128x128, FOV=40x40mm, NT=1).

Loss of BBB integrity and perfusion of cerebral cortex. An unbiased automated selection of cerebral cortex was performed via standard image processing methods. Thresholding and morphology were used to identify the full brain mask including ventricles, basal ganglia and the base of the brain. Morphological erosion and subsequent subtraction then extracted a region of interest, which was then halved to obtain the cortex used for analysis (see fig. 1). Data and results from the cortex voxels are presented as the fraction of the total number of voxels in each brain cortex selection.

A preliminary analysis of the dynamic MRI data sets using k-means clustering was performed to identify specific enhancement patterns associated with normal and infected animals. From this analysis, typical enhancement profiles were identified which could be divided into temporal regions. Consequently, data was divided into 2 major classes (non-enhancing and enhancing) and into 3 sub-classes of cortex ‘perfusion’: 1) well, 2) medium and 3) low-‘perfused’. Enhancing and non-enhancing voxels were classified according to whether significant T1 enhancement occurred within the voxel following gadolinium administration. Sub-classification of the 3 cortex perfusion profiles was performed according to the extent of T2* signal loss during the bolus passage. The T2* signal loss is dependent on the concentration and distribution of gadolinium within the tissue and will,
consequently, be dependent on a variety of physiological parameters including blood volume, flow and vascular permeability and dimensions. Hence, whilst the extent of signal loss not only reflects tissue perfusion, the approach provides a semi-quantitative parameter that gives an indication of how meningitis affects brain and muscle physiology.

*Muscle perfusion.* A class of well-‘perfused’ muscle was included in the analysis.

A reduced number of voxels in this cluster was interpreted as a reduction in muscular blood supply. Data for the infected animals are presented as a fraction of the corresponding control rat value at each time point. Failure to acquire complete data from one 48 hour control rat meant that correspondent infected rat data was unavailable.

**Measurement of ventricle-brain ratio (Fig. 2)**

Regions of interest around whole brain and lateral- and third ventricles were drawn on T2W images. The ventricle-brain ratio (VBR) was calculated as ventricular area divided by total brain area for all three coronal slices included. Measurements were performed blinded to all other data (HS).

**Histopathology**

After MR investigation, rats were euthanized with pentobarbital (200mg/ml) and perfused with 1.5% paraformaldehyde via the left ventricle of the heart. Brains were harvested and stored for 14 days in 1.5% PFA and frozen in n-hexane mixed and cooled with dry-ice. Two 45 µm thick coronal cryosections adjacent to each other, corresponding to the MR images obtained were stained with Hematoxylin Eosin (HE) in order to identify the nature of pathological features apparent in the MR images.

**Statistical analysis**

Data are presented as mean +/- SEM. Comparisons between controls and infected rats were performed by two-way ANOVA and P<0.05 was considered significant.

Due to the temporal development of the disease, two-way ANOVA was performed in 3 intervals for each dataset; 6 to 48 hours, 6 to 30 hours and 36 to 48 hours after infection.
Statistical comparisons between infected and control animals were only performed between MRI-generated datasets. Since muscle ‘perfusion’ data were presented as a fraction of results obtained in the correspondent control, a linear regression analysis of this dataset was performed.

To identify relationships between clinical, paraclinical and MRI data, Spearman Rank correlations were performed among meningitis rats in the terminal disease phase, 36 to 48 hours after infection. Since only a limited number of comparisons were performed, a P-value below 0.05 was also considered significant for correlation analysis.
Results

The evolution of pneumococcal meningitis in the experimental model (Fig. 3 and 4)

As shown in fig. 3, the increase in the paraclinical (CSF and blood bacterial counts, CSF WBC) and MRI data (ventricle-brain ratio (VBR), Apparent Diffusion Coefficient (ADC) and dynamic MRI (dMRI)) was non-linear as the disease progressed with time. The dual phase nature of the increases suggested that this model of the disease could be characterised by a Developing disease phase (up to and including 30 hours after infection) followed by a Terminal disease phase (from 36 to 48 hours).

Infection and inflammation (Fig. 3a and 3b). All CSF samples obtained from control rats were sterile. CSF and blood samples taken 6 to 48 hours after infection delineated the progression of meningitis with increasing CSF bacterial growth, secondary bacteremia and increasing CSF inflammation. Both CSF WBC and CSF bacterial counts peaked at 30 hours after infection. A plateau relieved by a downward slope in CSF WBC and CSF bacterial counts was observed in samples obtained from 30 hours onwards, declining until the final 48 hour study point. Blood bacterial counts showed that secondary bacteremia was present in one rat from 12 hours after infection and 3/4 rats at 24, 30 and 36 hours and 4/4 rats at 42 and 48 hours.

Development in disease scores (Fig. 3c and 3d). As expected, clinical disease severity increased in infected animals as the disease progressed. Subsequent to increasing clinical scores, motor performance, mainly ambulatory activity, deteriorated. Increasing inter-rat variation in both clinical and motor performance scores was observed as the infection developed, being marked from 30 hours onwards. In the terminal disease phase (36 to 48 hours) no further change in the scores was observed in either clinical or motor performance scores among infected rats. Infected rats imaged at 36, 42 and 48 hours after infection where comparable with respect to clinical disease and motor performance.
Only one rat, a priori designated for imaging in this late disease phase, was replaced.

Whilst this gave a slight bias in the experiment, this was outweighed by the advantage of having a full data set for each time point.

Following intracisternal inoculation of saline, clinical disease was not observed among these control rats although slight post-anaesthetic drowsiness (the Animal Inspectorate required animals to be transported to the MR centre whilst anaesthetised) accounted for a score of 1 in one control at 6 hours after inoculation.

**Ventricle-brain ratio – expansion of the lateral and third ventricle (Fig. 2, 3e and 4).**

The extent of ventricle expansion, determined as the ventricle-brain ratio (VBR), was comparable between controls and infected rats up to 30 hours post-infection at which time point 3 out of 4 infected rats had increased VBR compared to controls. When compared to controls, the VBR was significantly increased among infected rats in the total study period (6 to 48 hours, Two-way ANOVA, P<0.0001) as well as the terminal phase of meningitis (36 to 48 hours, P<0.0001), but not in the developing phase (6 to 30 hours, P=0.40).

In the terminal disease phase, a significant association was found between VBR and severity of clinical disease and motor disability (Spearmann rank, rho=0.62, P=0.024 and rho=0.57, P=0.04) whereas an inverse correlation was found between increased VBR and the fraction of enhancing brain cortex indicative of increased BBB permeability (rho= -0.73, P=0.01) as well as the muscle perfusion ratio (rho= -0.75, P=0.019).

**Loss of BBB integrity (Fig. 3f).** A measure of BBB integrity was obtained in 26 infected and 13 control rats. Increased BBB permeability, measured as the fraction of cortex voxels enhancing due to gadolinium leakage, was observed in one rat with meningitis as early as 6 hours after infection. From 36 hours onwards, all infected rats (n=11) had increased BBB permeability when compared to corresponding controls (n=5). The maximum fraction of enhancing voxels was found from 30 to 42 hours after infection. Compared to the control group, the total fraction of enhancing brain voxels was significantly increased
during the full course of disease as well as in the terminal phase (6 to 48 hours, Two-way ANOVA, \( P=0.0019 \) and 36 to 48 hours, \( P=0.019 \)), but did not quite reach significance during the developing phase (6 to 30 hours, \( P=0.06 \)).

*Cerebral cortex ‘perfusion’ (Fig. 3j, 3k and 3l).* ‘Perfusion’ data were obtained from 26 infected and 13 control rats. The marked shift towards enhancing cortex voxels, and thus increased BBB permeability, in rats with meningitis, was also apparent in the analysis of cortex ‘perfusion’ based on gadolinium bolus passage. In infected rats, the selected cortex region (fig. 1) shifted from non-enhancing well-, medium – and low-‘perfused’ cortex towards enhancing well-, medium-, and low-‘perfused’ cortex. This shift towards increased BBB permeability was significant in all 3 classes of ‘perfusion’ in comparison with the control groups (6 to 48 hours, Two-way ANOVA, well, \( P=0.017 \), medium, \( P=0.0018 \) and low, \( P=0.012 \)). However, the total fraction of well-, medium – and low-‘perfused’ cortex (non-enhancing + enhancing) did not change significantly among infected rats in comparison with controls in either the developmental stage (6 to 30 hours, Two-way ANOVA, well, \( P=0.97 \), medium, \( P=0.99 \), and low, \( P=0.98 \)), terminal stage (36 to 48 hours, well, \( P=0.78 \), medium, \( P=0.71 \), and low, \( P=0.2 \)) or full course of disease (6 to 48 hours, well, \( P=0.8 \), medium, \( P=0.76 \), and low, \( P=0.9 \)).

In the control group one 24 hour and one 42 hour rat had markedly reduced contrast delivery comparable to the most affected infected rats. The number of well-‘perfused’ voxels in jaw muscle was equally low in these control rats.

Muscle ‘perfusion’ (Fig. 3i). Data from 24/29 infected rats was successfully analyzed. The number of voxels representing well-‘perfused’ muscle in infected rats, relative to corresponding controls at each time point, showed a steady decline as infection progressed (Linear regression analysis, borderline significant, \( P=0.05 \)).
Apparent Diffusion Coefficient in cerebral cortex and basal ganglia (Fig. 3g and 3h).

Apparent Diffusion Coefficient (ADC) values were obtained in 28 infected rats and 13 controls. No significant differences were found between infected rats and controls in either developing or terminal disease stages (6 to 48 hours, Two-way ANOVA, P=0.47; 6 to 30 hours, P=0.12; 36 to 48 hours, P=0.67). However, increased variability in ADC values in cerebral cortex and basal ganglia was observed among infected rats in comparison with the highly uniform control group values. Three of four infected rats had increased cortex ADC values at 6, 12, 30 and 48 hours after infection, but only 2/4, 1/4 and 1/3 had increased ADC values at 24, 36 and 42 hours respectively.

A similar pattern was found in ADC values in basal ganglia, where 4/4 rats had increased ADC after 6 hours and 3/4 at 12, 30 and 36 hours after infection. Only 1/4 and 0/3 and 0/4 infected rats had increased ADC in comparison to controls at 24, 42 and 48 hours respectively. There were no significant differences between infected rats and controls (6 to 48 hours, P=0.38; 6 to 30 hours, P=0.062; 36 to 48 hours, P=0.47).

Brain injury (Fig. 5).

Focal injury to the brain parenchyma, other than hydrocephalus, was observed in four rats in the terminal stage of meningitis (two rats after 36 hours, one rat after 42 hours and one rat after 48 hours). Injury presented as a localized infarction, haemorrhage and abscess formation, the latter being readily observable on T1W post contrast images whereas the other lesions were smaller and less obvious on MR images.
Discussion

Few studies have combined MRI and experimental pneumococcal meningitis research [10]. The present study is the first to use minimally invasive in-vivo MRI methods to describe the development of brain pathoanatomy and pathophysiology in a meningitis model. Within 24 hours post-infection, MRI was able to detect physiological changes as the disease developed. In the terminal disease phase, the most marked results obtained with MRI were the dilated ventricles and increased BBB permeability. Cortex ‘perfusion’ and brain water distribution (ADC) also changed but appeared to be subject to greater variation making interpretation of the data less obvious. Regions of well-
‘perfused’ muscle declined as infection progressed indicative of secondary septicaemia. Hydrocephalus is a well-known complication of bacterial meningitis associated with poor outcome and brain injury [15-17], and preliminary studies directed at reducing intracranial pressure appear promising [18]. This study describes a marked expansion of ventricles that appears to develop, in the terminal disease stage [10] in close association with development of severe clinical disease and deteriorating motor performance. This is in agreement with recent findings of a close association between clinical disease score and intracranial pressure [19].

A hallmark of bacterial meningitis is the loss of BBB integrity allowing CSF leukocyte accumulation and diffusion of water and plasma constituents into brain parenchyma. In the present study, permeability of the vascular barrier increased markedly as meningitis progressed. The loss of BBB integrity appeared to be an efficient disease biomarker, making it possible to identify infected and control animals at early time points after infection. In comparison to methodology previously applied to investigate BBB integrity such as Evans Blue staining [20], MRI enabled quantification and visualization of areas with increased BBB permeability that were confined to the outer layers of cerebral cortex (see fig. 1). The close association between increased VBR and low fraction
of enhancing cortex and thus a low BBB permeability suggests that expansion of the ventricles may influence cortex ‘perfusion’ and via physical compression alter, for example, interstitial volume. This may account for the observed changes in brain water distribution and contrast agent kinetics even though the present study failed to identify the association between the two parameters.

Brain water content has in previous experimental meningitis studies been shown to increase as a consequence of the infectious and inflammatory response [21-23]. To some extent, this is in accord with our measurements of ADC, reflecting altered water distribution in the extracellular compartment (vasogenic oedema [24]) in cerebral cortex and basal ganglia in the majority of infected rats in the developmental stage of meningitis. Normalisation, or decrease, of ADC was observed in the terminal stage of meningitis and could be related to the concomitant ventricle expansion, as has previously been shown [25]. In the early stages of meningitis, increased brain water content may reflect increased blood volume [26]. This would be in agreement with previous findings of increased cerebral blood flow in early meningitis and changes in ADC due to alterations in blood flow- and volume [21;27]. This study has not investigated the distribution of water between tissue and ventricles. Future studies measuring ventricular size and ADC in combination with wet/dry weight analysis would provide further mechanistic insight into this characteristic of the disease.

Our semi-quantitative measurement of ‘perfusion’ in cerebral cortex was not subject to any significant overall changes in the distribution of well-, medium or low ‘perfused’ areas in the cortex, whereas the area of well-‘perfused’ muscle declined as infection progressed. The latter being in agreement with studies of muscle perfusion in septicaemia showing areas of well perfused muscle to decrease as septicaemia worsens [28-30]. Previous studies have argued that cerebral perfusion is compromised in bacterial meningitis as a consequence of systemic disease, brain oedema, and raised intracranial
pressure [3;31;32]. Our experimental data do not support this as a general assumption, since we found a large variation within ‘perfusion’ categories in the terminal phase of meningitis with seven of 15 rats between 30 and 48 hours after infection presenting an increased fraction of low perfused brain areas (Fig.3L). Importantly, our results on cerebral ‘perfusion’ were limited to the cortex that has previously been shown experimentally to be more well ‘perfused’, even in severely ill subjects [23], and in patients to be subject to great variation and regional differences [33]. It is important to state that the measure of ‘perfusion’ reported here is, as described above, dependent on a number of other physiological factors and not just perfusion.

Several limitations in the presented study should be considered. Firstly, biological variation in disease development and the limited numbers of animals used makes temporal comparison sub-optimal. Whilst the sacrifice of rats at designated time points was performed to ensure histopathological evaluation, this limited the study of pathophysiological events preceding a poor outcome. Imaging of rats on more than one occasion would have significantly improved analysis of interrelationships.

The dMRI measurements only provide a semi-quantitative measure that reflected perfusion. Quantitative perfusion measurements could be performed following sequence optimisation to acquire kinetic changes in relaxation times following gadolinium administration. The gadolinium concentration could then be fitted to appropriate kinetic models to obtain measurements of vascular permeability, extracellular volume and perfusion. Also, ADC measurements may be improved by introducing measurement of water motion in three or even more planes as compared to the single plane performed here. In addition, the necessary use of a total anaesthesia during scanning sessions affects pathophysiological parameters and result in changes in vasodilatation, blood pressure, blood supply or ventilation [34] in a disease-dependent way.
Even though our study was performed using a dedicated 4.7 Tesla animal scanner, the resolution was not optimal and small lesions in the cerebral cortex were not readily recognized as areas with lesions until after the histopathological evaluation of the corresponding specimen. Image quality would be improved by incorporating at least one sequence with very high resolution or working at higher magnetic field strengths.

Further studies incorporating significant interventions aimed at altering the course and pathophysiology of meningitis is required to validate the presented data and its interpretation. It is expected that MRI will prove to be an essential tool for the development and assessment of new adjunctive treatments. The ability of advanced MR methods able to monitor changes in brain pathoanatomy and pathophysiology should facilitate the identification of parameters related to improved outcome, assisting in the development of new and successful therapeutic approaches.
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5. Ostergaard, C., R.V. Yieng-Kow, T. Benfield, N. Frimodt-Moller, F. Espersen, and J.D. Lundgren. 2000. **Inhibition of leukocyte entry into the brain by the selectin blocker fucoidin decreases interleukin-1 (IL-1) levels but increases IL-8 levels in cerebrospinal fluid during experimental pneumococcal meningitis in rabbits.** Infect. Immun. **68**: 3153-3157.


Figure 1. Dynamic MRI data analysis:

(a) Categorization of dMRI profiles: Typical data sets showing dynamic MRI signal intensity profiles categorized into non-enhancing and enhancing parent classes and well ‘perfused’, medium ‘perfused’ and low ‘perfused’ sub classes. Enhancing and non-enhancing pixels were identified according to whether curves returned to baseline (non-enhancing) or increased (enhancing) above baseline after the signal intensity reached its minimum value due to T2* losses. The extent of T2* induced signal loss determined the pixel sub-class and was, subsequently, assigned a colour.

(b) Automatic cortex region of interest selection: Proton image (i) also shown with a brain mask (ii) calculated using thresholding and morphology. Morphological erosion of the brain mask (blue) yielded a cortex mask (iii) enabled an automatic, unbiased selection of a cortex region of interest (yellow). In addition, maps of the parent classes were obtained as shown in (iv) showing pixels that underwent contrast enhancement (blue) and those that failed to enhance (orange).

(c) Cortical regions of interest: Cortex brain masks show the distribution of well (red), medium (orange) and low perfused (yellow) non-enhancing voxels. Green voxels, dominant in outer cortex layers from rats with meningitis, show enhancing voxels indicative of blood brain barrier breakdown.

Figure 2. Hydrocephalus in experimental meningitis. T2W images showing a control rat (a) and two infected rats imaged at 36 hours (b) and 42 hours (c) presenting significant dilation of lateral and third ventricles (outlined in red) indicative of hydrocephalus. Brain-ventricle ratio (VBR) in a, b and c was 0.025, 0.076 and 0.085, respectively.
**Figure 3. Development of pneumococcal meningitis.** Graphs (a) to (l) show the development and changes in all included study parameters from 6 to 48 hours after inoculation in infected (n=29, solid median line) and control rats (n=13, dashed median line). Graph (a) and (b) show median and interquartile range of bacterial counts and WBC counts. Graph (c) and (d) show the steady worsening of clinical disease and deteriorating motor performance among infected animals. The ventricle-brain ratio (VBR) in (e) was subject to marked development among infected rats from 30 hours after infection and all infected rats had increased VBR from 36 hours onwards (P<0.0001). The number of enhancing voxels (f), indicative of BBB breakdown, was significantly increased among meningitis rats (6 to 48 hours, P=0.0019). Graphs (g) and (h) illustrate comparable ADC values in cortex and basal ganglia among infected rats with increased variation around the mean values from the control group until 36 hours after infection (P>0.05). Graph (i) shows decreased areas of well ‘perfused’ muscle (no. of voxels) in infected rats presented as a fraction of the value in corresponding control animals at each time point (P=0.05). Graphs (j), (k) and (l) show the total number of voxels (enhancing + non-enhancing) in each ‘perfusion’ category (P>0.05).

**Figure 4. Disease evolution visualized using MRI**

Pre- and post contrast T1W images (a, b), T2W (c) images together with equivalent histological slices (d) illustrate the evolution of the disease. Postcontrast T1W images were used to identify meningeal enhancement. Meningeal enhancement could visually be graded as: 0) No enhancement, 1) Thin brim of enhancement, 2) Thick brim of enhancement and 3) Diffuse enhancement with unclear borders towards outer cortex layer. T2W images (c) clearly show increasing ventricular size that was also apparent in the histological slices prepared using standard processing methods.
Figure 5. Infection induced brain injury. Example of an infected rat (48 hours) with a
cortical abscess, confirmed histopathologically (a and b), apparent in post-contrast T1W-
and T2W images (c and d). The damaged area identified with histology covered only 1/3
of the area with contrast enhancement.
Figure 1

(a) Signal intensity (a.u.)

- Non-enhancing
- Enhancing

Image number

(c) Infected

- Well ‘perfused’
- Medium ‘perfused’
- Low ‘perfused’

Control

- 12 hours
- 24 hours
- 36 hours
- 48 hours

(b) (i) (ii)

(iii) (iv)
Figure 2
Figure 3
Figure 4
Figure 5
Additional files provided with this submission:

Additional file 1: table 1.doc, 41K
http://www.biomedcentral.com/imedia/1495893924135985/supp1.doc