The inflammatory cellular constituents of foetal and infant leptomeninges – a survey of hospital-based autopsies without trauma

Abstract
Introduction: We investigated the possible association of leptomeningeal inflammatory infiltrates and iron deposition in neonates and infants, with the objective of deriving a baseline for reference in forensic cases.

Methods: Leptomeninges derived from non-forensic deaths with atraumatic, natural causes was studied. Because of the vastly dissimilar neuroanatomy between newborn infants and older ones, 33 cases were divided into two groups, according to set age groups. Inflammatory cells and iron levels in these were quantified.

Results: In group 1 there was a correlation between an increased number of inflammatory cells and the presence of subdural or subarachnoid haemorrhages. Inflammatory cells, albeit reduced in number, were also present in a number of cases in the absence of subdural or subarachnoid haemorrhages. Iron was found in the leptomeninges in several cases in similar quantities, even those without recent haemorrhage. Overall and within the two subgroups, ranges and means of the counts were wide and not significantly different.

Conclusion: These findings suggest that inflammatory cells and iron in the leptomeninges can be found in a number of natural and non-traumatic conditions. Further, two cases with no reported neuropathology demonstrated the presence of inflammatory cells and iron. Thus, cautious interpretation of neuropathology found in paediatric forensic cases is recommended.

Introduction
The leptomeninges are composed of the pia and arachnoid mater connected by strands termed arachnoid trabeculae. In the young, the leptomeninges are clear, but they become gradually thickened with age. The leptomeninges and dura mater have been traditionally thought to contain few, if any, inflammatory cells, and any increase in cellularity is potentially equated to a pathologic condition, including inflicted trauma. At present, we do not know what constitutes ‘normal’ cellularity of infant leptomeninges and if they should be present at all in non-forensic settings. However, when indeed present under suspicious circumstances, they are often linked to inflicted trauma, such as in cases of the ‘shaken baby syndrome’.

In order to recognise and characterise the pathologic findings in infant brains, it is important to have an understanding of the normal constituents of the various intracranial compartments. While some studies in the past, largely in rodent pups, have sought to evaluate and characterise the leptomeningeal cellular constituents, until now a rigorous analysis of the inflammatory cellular constituents of the leptomeninges has not been performed in human late-foetal and infant brains. This characterisation will serve as a baseline for comparison with brains of similarly aged children in forensic settings. Therefore, in addition to determining the inflammatory cellular composition and iron quantities of foetal and infant leptomeninges associated with natural disease processes, and in
the absence of physical trauma beyond that accompanying vaginal birth, this study aims to formulate a basis of comparison of leptomeningeal cellular constituents in forensic settings, based on rigorous histological analyses of hospital-derived autopsies.

Materials and methods

Subject selection
Thirty-three foetal and infant autopsies in which neuropathologic examinations had been performed at Stanford University Medical Center/Lucille Packard Children’s Hospital were identified utilising the department of pathology database program. The cases for study were chosen in concert with the attending neuropathologist responsible for rendering the original diagnoses. The criteria for study inclusion were cases between 2005 and 2008, the age bracket of between late third trimester and one year of post-natal life, all of which have samples harvested from at least two different brain sites including the cerebral cortex, cerebellum and brainstem. Three cases within the 33 total cases that did not conform to these criteria were included.

Leptomeningeal sample selection
Each sample slide was screened on microscopy by the attending neuropathologist and only sections of brains reflecting a wide (±5.0mm) sampling of the leptomeninges were chosen.

Sample fixing, staining and immunohistochemistry
Slides of routinely processed formalin-fixed, paraffin-embedded sections in each case were prepared and stained with antibodies to CD45 (dilution 1:100), CD68 (dilution 1:100) and CD163 (dilution 1:200).

CD45, also known as leucocyte common antigen, is uniquely expressed on the surface of all leucocytes and their progenitor cells – these include neutrophils, eosinophils, basophils, lymphocytes and monocytes. CD68 is expressed in monocytes and macrophages and thus represents reactive microglial activity. CD163 was used as an additional stain for cells of monocyte/macrophage lineage.

Immunoperoxidase staining was performed, following microwave antigen retrieval in citrate buffer at pH 6, on an automatic stainer (Dako Autostainer, Universal Staining System). Iron was detected in sections utilising the standard Perl’s staining method.

Examination of samples
In order to reduce inter-observer variation, the number of variously immunoreactive cells was quantified by a single observer and representative slides reviewed for accuracy by a second observer. At a microscopic magnification of x20, immunoreactive cells within leptomeninges were counted and recorded.

As the length of leptomeninges evaluated varied between slides, the length of leptomeninges scored was measured in millimetres and results recorded as immunoreactive cells/millimetre. Only leptomeninges on gyral surfaces were scored as it was impossible to measure the depth of layers that were in the sulci.

Table 1: Site and range, mean and standard deviations of lengths of leptomeninges in all cases.

<table>
<thead>
<tr>
<th>Sites</th>
<th>No. of sites sampled</th>
<th>Leptomeningeal length (mm)</th>
<th>Mean (mm) +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>39</td>
<td>5-41</td>
<td>18.8 +/- 10.5</td>
</tr>
<tr>
<td>Brainstem</td>
<td>37</td>
<td>10-83</td>
<td>28.7 +/- 17.0</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>30</td>
<td>10-47</td>
<td>20.3 +/- 11.1</td>
</tr>
</tbody>
</table>

Analysis of results

The samples were divided into infants who died beyond 33 post-natal days up to one year (group 1) and newborns who survived up to 33 days (group 2) – these represent pre- and post-natal leptomeninges.

The mean immunoreactive cells/millimetre for CD45, CD68, and CD163 was calculated for groups 1 and 2. Iron was recorded as being either present or absent.

No further statistical analysis was performed beyond demonstrating the mean values in the form of bar charts.

Results

Demographics

Of the 33 cases, 16 were male and 17 female. Thirteen were born via vaginal delivery and 19 via Caesarean section. The mode of delivery of one case was not available. Seventeen cases involved infants who died beyond the post-natal age of 33 days (group 1), and 16 cases represented either foetuses or newborns who survived up to 33 post-natal days (group 2). One child (number 7) who survived to 16 months of age, was included in group 1. There were two cases (numbers 25 and 30) involving foetuses in the 26th and 28th weeks of gestation, which were included in group 2.

The general autopsy and neuropathology findings of both groups overlapped, and these included congestive heart failure, Noonan’s syndrome, microencephaly, and pontosubicular neuronal necrosis.

Table 1 demonstrates the number of slides from each brain region and the range of length, mean and standard deviation of associated leptomeninges scored per slide. Overall, 39 sites were sampled from cerebral cortices, 37 from brainstems, and 30 from cerebella. These involved leptomeningeal lengths of between 5mm and 83mm.

Group 1

Looking specifically at the cases in group 1 (Table 2 and Figure 1), four had congenital heart disease (CHD) and two were diagnosed with Noonan’s syndrome. The mean density of CD45, CD68 and CD163 immunoreactive cells per mm of leptomeninges of the former group was 14.4, 17.5 and 17.9 cells/mm, respectively, and of the latter group was 22.3, 18.6 and 21, respectively. Two cases in which sepsis or significant infection was documented (cases 22 and 8) had mean CD45, CD68 and CD163 counts of 19.3, 27.4 and 34.1 cells/mm, respectively. In group 1, 11 of 17 cases had some form of hypoxic/ischaemic event (pontosubicular neuronal necrosis, hypoxic-ischaemic encephalopathy, infarction, periventricular

Table 2: Immunoreactive cell counts for CD45, CD68, and CD163.

<table>
<thead>
<tr>
<th>Sites</th>
<th>CD45 (cells/mm)</th>
<th>CD68 (cells/mm)</th>
<th>CD163 (cells/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>5-22 (14.4)</td>
<td>10-22 (17.5)</td>
<td>10-22 (17.9)</td>
</tr>
<tr>
<td>Brainstem</td>
<td>12-24 (22.3)</td>
<td>14-26 (18.6)</td>
<td>13-22 (21)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>10-20 (19.3)</td>
<td>14-25 (27.4)</td>
<td>12-22 (34.1)</td>
</tr>
</tbody>
</table>
leukomalacia, hypoxic-ischaemic changes). The mean CD45, CD68 and CD163 positive cells/mm for this subgroup was 11.4, 15.5 and 19.0 cells/mm, respectively. Cases in the hypoxic/ischaemic subgroup incorporated cases in each of the other subgroups. Two cases had evidence of organising haemorrhages (epidural, subdural or subarachnoid; cases 26 and 28). The mean CD45, CD68 and CD163 cell counts/mm in this subgroup were 33, 35.8 and 40.7, respectively. These three cases had the highest mean CD45, CD68 and CD163 counts within group 1. These three cases also contained cells in the leptomeninges with stainable iron in at least one section. An additional 12 cases in group 1 demonstrated some degree of iron staining in at least one brain section.

Table 2: General autopsy and neuropathologic findings for infants with a post-natal age greater than 33 days (group 1).

<table>
<thead>
<tr>
<th>Case No</th>
<th>General autopsy findings</th>
<th>Neuropathology findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chronic aspiration/pneumonitis with MOSF</td>
<td>Oedema with herniation; HIE</td>
</tr>
<tr>
<td>4</td>
<td>Noonan’s syndrome</td>
<td>Micrencephaly; Pontosubicular neuronal necrosis</td>
</tr>
<tr>
<td>5</td>
<td>Trisomy 21 with CHD</td>
<td>Haemorrhagic infarct (left periventricular)</td>
</tr>
<tr>
<td>7</td>
<td>CHD</td>
<td>Micrencephaly</td>
</tr>
<tr>
<td>8</td>
<td>Interstitial pneumonitis (cultures negative)</td>
<td>PVL</td>
</tr>
<tr>
<td>9</td>
<td>Heterotaxy-asplenia syndrome</td>
<td>Micrencephaly; Multiple cortical and WM infarcts</td>
</tr>
<tr>
<td>11</td>
<td>Congenital pulmonary malformation</td>
<td>Multifocal acute HI changes</td>
</tr>
<tr>
<td>12</td>
<td>Pulmonary hypoplasia</td>
<td>Remote and focal acute HI changes</td>
</tr>
<tr>
<td>14</td>
<td>None (brain only)</td>
<td>Agenesis of CC</td>
</tr>
<tr>
<td>17</td>
<td>CHD with infarction</td>
<td>None</td>
</tr>
<tr>
<td>22</td>
<td>Cardiomyopathy; Sepsis with MOSF</td>
<td>Multiple cerebral infarctions</td>
</tr>
<tr>
<td>23</td>
<td>Foetal hydrops</td>
<td>PVL; Right occipital infarction</td>
</tr>
<tr>
<td>26</td>
<td>Noonan’s syndrome</td>
<td>Micrencephaly; Hypomyelination; Organising SAH, SDH</td>
</tr>
<tr>
<td>27</td>
<td>Liver failure</td>
<td>Metabolic astrogliosis; Neuronal pyknosis; bilateral subdural membranes</td>
</tr>
<tr>
<td>28</td>
<td>RSV bronchiolitis; Pneumonia</td>
<td>Remote occipital infarct; Subacute EDH</td>
</tr>
<tr>
<td>33</td>
<td>CHD</td>
<td>Cystic infarct (frontal)</td>
</tr>
</tbody>
</table>

CC – corpus callosum; CHD – congenital heart disease; EDH – epidural haemorrhage; GM – germinal matrix; HI – hypoxic/ischaemic; HIE – hypoxic/ischaemic encephalopathy; MOSF – multiple organ system failure; PVL – periventricular leukomalacia; RSV – respiratory syncytial virus; SAH – subarachnoid haemorrhage; SDH – subdural haemorrhage; WM – white matter

![Figure 1: Mean immunoreactive cells in cerebral cortex, brainstem and cerebellum (group 1).](image.png)
Looking specifically at the cases in group 2 (Table 3 and Figure 2), eight cases had CHD. The mean density of CD45, CD68 and CD163 immunoreactive cells/mm was 9.4, 16.6 and 23.2, respectively. Two cases in which sepsis or significant infection was documented had mean CD45, CD68 and CD163 counts of 31.8, 28 and 33 cells/mm, respectively. In group 2, 10 of 16 cases had some form of hypoxic/ischaemic event as noted above with CD45, CD68 and CD163 positive cells of 18, 20.5 and 24.6 cells/mm, respectively. Seven cases had some form of haemorrhage involving the dural surface or extending into the subarachnoid space. The number of CD45, CD68 and CD163 immunoreactive cells/mm in this subgroup was 13.5, 25.5 and 32.4 cells/mm, respectively. Five cases of seven
in this subset with associated haemorrhage also had evidence of iron staining. Two cases with no reported neuropathologic findings had CD45, CD68 and CD163 counts of 5.9, 6.2 and 11.5 cells/mm, respectively. An additional eight cases in group 2 demonstrated some degree of iron staining in at least one brain section.

Cases without reported neuropathology
In two cases without neuropathologic abnormalities, all classes of inflammatory cells were found in the leptomeninges, albeit at relatively low numbers (case 17 of group 1 and case 6 of group 2).

Iron findings
Of the 19 cases in which Caesarean sections were performed, 16 had positive iron findings, whereas eight cases of the 13 vaginal births were positive for iron. Four cases of each mode of delivery reported haemorrhage-related neuropathologic diagnoses. Fifteen cases of Caesarean section births were associated with some form of hypoxic/ischaemic event in contrast to seven cases involving vaginal births.

Discussion
In the current study we found the presence of inflammatory cells in the leptomeninges, both overall and when segregated into two groups by age (foetal and early post-natal vs. infants beyond 33 days post-natal life) and by anatomic and neuropathologic conditions. A notable finding is that even in foetuses and infants with no neuropathologic abnormalities, inflammatory cells, and occasionally iron, were identified in the leptomeninges. This is in contrast to the widely held belief that the leptomeninges should be largely devoid of inflammatory cells and iron in children with no reported neuropathology. In the infant group, in fact, there was a positive association between the degree of leptomeningeal inflammation and the presence of epidural haemorrhage (EDH), subdural haemorrhage (SDH) or subarachnoid haemorrhage (SAH) (cases 26 and 28).

In group 1 the presence of some form of chronic haemorrhage was associated with higher numbers of inflammatory cells compared to those without such haemorrhage. Caesarean section deliveries and vaginal births were associated with a variety of anatomic and neuropathologic diagnoses, with hypoxic/ischaemic events commonly found in both modes of delivery. Both modes of delivery also demonstrated iron deposition in the leptomeninges and haemorrhage-related neuropathology. Accordingly, it appears that the presence of iron in the leptomeninges does not necessarily equate to traumatic haemorrhage but may be found in completely naturally occurring processes, and occurs irrespective of the mode of delivery. There also seemed to be no recurring pattern allowing us to associate the presence of iron to a single anatomic or neuropathologic diagnosis.
There are multiple mechanisms that allow the brain to sense inflammatory signals from systemic circulation, including interacting with circulating molecules in areas in the brain devoid of the blood-brain barrier. Microglial cells seem to migrate from the germinal matrix to the cortical layers. Early migration of microglia from the blood-brain barrier. Microglial cells seem to migrate from the germinal matrix to the cortical layers. Early migration of microglia from the blood-brain barrier. Microglial cells seem to migrate from the germinal matrix to the cortical layers.

The function of resting microglia under normal conditions is unclear. In pathologic conditions, these microglial cells are rapidly activated and proliferate. Animal studies have demonstrated that inflammation, either introduced systemically or within the brain, causes microglial activation along with cytokine release. Accordingly, there is evidence to suggest that infection distant from the brain may damage developing foetal brain. The activation of neuroinflammatory responses may also sensitize the brain to the damaging effects of other insults, such as hypoxia/ischaemia, and amplify the effects of the latter.

Thus there are multiple possible reasons to account for the presence of inflammatory cells within the leptomeninges early in gestation in humans outside of inflicted trauma.

Conclusion
We conclude that a number of patients with various natural disease processes in this hospital-based population had significant numbers of CD45, CD68 and CD163 immunoreactive cells and iron in the leptomeninges overall and when segregated by age, and anatomic and neuropathologic diagnoses. Although we studied a larger number of cases than the dura study by Croft et al., our numbers of total cases are relatively small, particularly in consideration of the varied anatomic and neuropathologic diagnoses. Our cases were derived only from hospital autopsies and these observations require comparison to actual forensic cases involving both traumatic injuries and natural disease processes. Clearly the presence of inflammatory cells and iron in the leptomeninges can occur commonly, and in significant numbers, in non-traumatic neuropathologic conditions. It has not been possible to assign a value to which the presence of inflammatory cells would be deemed arising from suspicious events. Thus, these findings support the recommendation of cautious interpretation of the findings of leptomeningeal inflammation and iron in forensic cases.

References