Diaphragmatic Pathology: A cause of clinically unexplained death in the perinatal/paediatric age group

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ABSTRACT

Sudden unexpected death in infancy and childhood requires a 'full' post-mortem investigation. Guidance from the Royal College of Pathologists recommends sampling of all the major organs. However, the diaphragm does not feature in this or in most lists of routine histology. Our aim is to emphasize the importance of sampling the diaphragm for histological examination during autopsy. We describe three autopsy cases of clinically unexplained death in the perinatal and paediatric age group that showed significant pathology of the diaphragm. In Case 1, a previously healthy five-year-old girl collapsed suddenly and died four days later. In Case 2, an eight-month-old infant had repeated episodes of respiratory arrest that culminated in death. Autopsy demonstrated a predominantly diaphragmatic myositis. In Case 3 a female neonate had a respiratory arrest three days after birth and died less than a month later. Autopsy showed multiple large calcified necrotic fibres in the diaphragm.

The diaphragm is seldom sampled at autopsy. In the first two cases a predominantly diaphragmatic myositis was either the direct or underlying cause of death. In the third case long-standing diaphragmatic pathology of uncertain cause may have contributed to the original respiratory arrest.

Had the diaphragm not been examined histologically, the cause of death would have remained unascertained in these cases. In cases of sudden death in infancy and childhood, failure to reach a diagnosis may lead to undue suspicion falling upon the child's carers. This underscores the need for full histology at post-mortem in child deaths, including diaphragmatic sampling.

INTRODUCTION

The diaphragm is one of the largest muscles of the body and is by far the most important of the respiratory muscles (Sharp, 1980). It has been described as second in importance only to the heart in maintaining life (Macklem, 1980). Significant diaphragmatic pathology could cause derangement of ventilatory function with consequent respiratory failure and thereby play a causative or contributory role in cases of clinically unexplained death. We describe three autopsy cases of clinically unexplained death in the perinatal/paediatric age group that had significant pathology of the diaphragm.

MATERIALS AND METHODS

In all three cases, all the organs were sampled for histology including the diaphragm, skeletal

muscle, and myocardium. The tissue was fixed in 10% formol saline and paraffin embedded. 3 μm thick sections were cut and stained with haematoxylin and eosin (H&E, Harris). In addition, immunohistochemistry was performed for CD20 (clone L26, Dako), CD68 (clone PGM1, Dako), and CD3 (clone PS1, Novocastra) on the diaphragm, psoas and myocardium in all three cases. In the case of CD20 and CD68 the tissue was pressure-cooked in 0.1 mol citrate buffer at full power for three minutes, and in that of CD3 was microwaved in 0.1 mol citrate buffer for 30 minutes for antigen retrieval. The diaphragm in Case 1 was examined for viral inclusions under the electron microscope and was tested by PCR for enteroviral DNA. The diaphragm in Case 3 was stained with von Kossa and Alizarin red to demonstrate stainable calcium.

Clinical details

Case 1

A five-year-old black girl who had been growing and developing normally, was admitted to hospital complaining of feeling unwell with chest and abdominal pain and mild difficulty walking, all of recent onset. She was discharged following a full examination as nothing significant was found.

On arrival at home she complained of further pain and collapsed. She was resuscitated in the hospital where her pH was 6.59 and Hb 10.7 g/dl, but all other investigations were normal. She was transferred to the intensive care unit (ICU) but the cause of collapse remained unascertained and she died four days after her first admission.

Case 2

An eight-month-old female infant, two months prior to her death, had an infection of presumed viral aetiology. Subsequently she had repeated brief admissions for transient episodes of respiratory arrest. Following a final collapse she was on a ventilator for eight days prior to death. All investigations during life were negative. Following the death, the case was subject to a full investigation by police and social services. However, no evidence was found to indicate that the child had been abused and the subsequent inquest found that death had been due to natural causes. The child's siblings were not placed on the child protection register.

Case 3

A female neonate, born by normal vaginal delivery at term with normal Apgar scores, was found floppy in her cot three days after birth and admitted to the ICU in respiratory arrest. On admission she was pink but floppy, with palpable femoral pulses, no rashes, flat anterior fontanelle and unresponsive. CT and MRI scans showed features of hypoxic-ischaemic encephalopathy. She died 24 days later having remained in the ICU.

RESULTS

Case 1

Histological examination of the diaphragm revealed a widespread severe mononuclear inflammatory cell infiltrate in the diaphragm. There was quite extensive necrosis and phagocytosis of muscle fibres (Figure 1a). Immunostaining for CD68 showed the great majority of the inflammatory cells to be macrophages (Figure 1b). A small population of CD3 positive T-cells was also present and there were occasional CD20 positive B-cells. The myocardium was largely normal although there were very occasional tiny foci of mononuclear cells, which may represent a mild myocarditis, however, no muscle cell necrosis was identified. The psoas showed a single focus of muscle cell necrosis and inflammation and there was a mild myositis in the neck muscles.

Case 2

Histological examination of tongue, skeletal and diaphragmatic samples was abnormal, though the extent of the pathology was very variable, being most severe in the diaphragm. Here much of the muscle had been replaced by collagen and inflammatory tissue, which appeared to be of variable age. Some areas contained numerous polymorphs. There was compensatory hypertrophy in surviving muscle. A few foci of calcification were noted. Extensive areas of collapse were seen in the lungs. The brain showed massive neuronal devastation, the vast majority being shrunken,

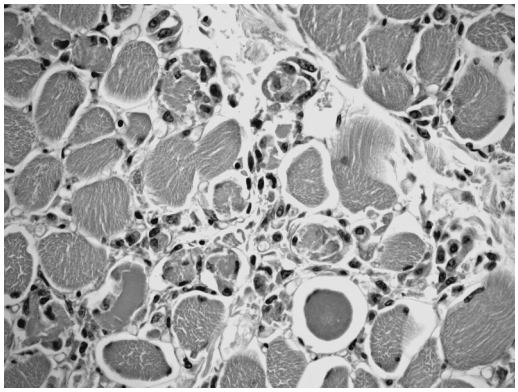

Figure 1a. Case 1 Diaphragm showing extensive inflammatory cell infiltration with phagocytosis of myocytes (H&E).

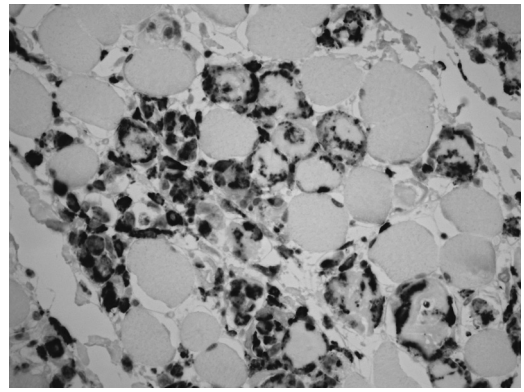

Figure 1b. Case 1 Diaphragm CD68 immunostaining shows the majority of cells in the infiltrate are macrophages.

eosinophilic and containing pyknotic, sometimes fragmenting nuclei. On immunohistochemistry, both diaphragm and psoas showed a moderately severe inflammatory infiltrate – more marked in the diaphragm – composed largely of CD68 positive macrophages with scanty clusters of CD3 positive T-cells and the very occasional CD20 positive B-cell. The heart was unremarkable.

Case 3

Histology of the lung showed bronchopneumonia. Multiple, often large, calcified necrotic fibres, together with sparse foci of fibre regeneration were seen in the diaphragm; no endomysial/perifascicular fibrosis and no significant inflammation was seen. Scattered CD68 positive macrophages and multinucleated giant cells were seen mainly associated with calcified fibres (Figure 2). Scanty CD3 positive T-cells and very occasional CD20 positive B-cells were also present. There was extensive encephalomalacia of almost the complete brain, with a partly preserved brainstem containing numerous haemosiderin-laden macrophages and numerous encrusted neurones. Also observed was an organizing thrombus of the transverse sinus.

DISCUSSION

Death of an infant or young child, following unexplained and repeated episodes of respiratory arrest, would be viewed by many pathologists as highly suspicious, raising the spectre of repeated imposed upper airways obstruction and non-accidental injury. Whilst deliberate harm to the baby or child should, of course, be considered in these circumstances, it is essential that as many natural causes as possible are excluded at post-mortem to avoid unjustified investigation and prosecution of the parents or other carers. In Cases 2 and 3 described in this paper, the history of unobserved, resuscitatable respiratory arrest, recurrent in Case 2, could be consistent with imposed upper airways obstruction. In Case 2 this led to a police investigation, but no evidence of deliberate harm could be

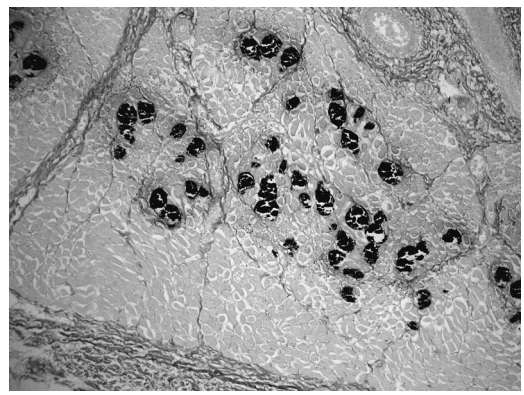

Figure 2. Case 3 Diaphragm showing numerous calcified myocytes (von Kossa).

identified. However, in both these cases, together with Case 1, histological examination of the diaphragm demonstrated extensive pathological abnormality, which may have accounted for the deaths.

Acute respiratory distress or failure due to diaphragmatic dysfunction has been described in polymyositis (Schiavi et al.,1984; Sano et al., 1994; Dauriat et al., 2002) congenital myotonic dystrophy (Yong et al., 2003), poliomyelitis and severe Guillain-Barré syndrome. However, diaphragmatic pathology as a cause of clinically unexplained death is uncommon, with only a few cases reported in the English literature to our knowledge. De Reuck et al. (1977) reported the case of a 20-month-old girl who presented with acute upper respiratory infection, followed by respiratory distress and death; autopsy demonstrated an acute viral polymyositis with predominant diaphragmatic involvement. Blumbergs et al. (1984) described a 36-year-old woman who presented with increasing respiratory distress, terminating in ventilatory failure and death due to paralysis of respiratory muscles; post-mortem revealed polymyositis with preferential involvement of the diaphragm.

In the first two cases considered in this article, histology showed a preferentially diaphragmatic myositis. Although there was much milder involvement of the other muscles (possibly including the heart in Case 1), unusually, the brunt of the inflammatory process was borne by the diaphragm. Whilst the clinical history and histological findings argue in favour of a viral etiology, this could not be confirmed despite electron microscopy and PCR in Case 1. However, it is well recognized that satisfactory evidence of viral etiology is available in very few cases of presumed viral myositis (Carpenter et al., 2001a). Whatever the cause of the muscular pathology in our first two cases, the extent of inflammation in the diaphragm is such as to explain the short course to death in Case 1 and the clinical history of recurrent attacks of cyanosis and respiratory arrest in Case 2. Presumably severe inflammation of the diaphragm caused diaphragmatic muscle fatigue and resulted in respiratory arrest and eventual death.

Helliwell et al. (1991) described a necrotising myopathy in critically ill adult patients who spent substantial periods in the intensive care unit receiving artificial ventilation. We believe that this is unlikely to be the explanation of the diaphragmatic pathology in the cases reported here. In Case 1 the presentation was acute with only about four days spent in the intensive care unit. In Case 2, although there were repeated brief admissions for transient episodes of respiratory arrest only the last eight days before death were spent in the intensive care unit. In the cases described by Helliwell (1991), a prominent inflammatory infiltrate was reported in only one of their cases with infective vasculitis, whereas inflammation was prominent in our Cases 1 and 2. Calcification, as seen in Case 3, was not described. There are no reports of intensive-care related myopathy in babies or children.

In Case 3, it is impossible to be certain whether the diaphragmatic calcification preceded or succeeded the initial respiratory arrest. It can be argued that the diaphragmatic calcification was a consequence of hypoxic/anoxic injury caused by the respiratory arrest. However, the absence of hypoxic damage to the other organs barring the brain militates against this. We speculate that pre-existing diaphragmatic pathology may have resulted in respiratory arrest and subsequent hypoxic brain injury. This raises the question as to the cause of the diaphragmatic calcification. It may be that the calcification was a consequence of the pre-existing diaphragmatic pathology. We cannot exclude the possibility that the findings were a result of prolonged intensive care as described by Helliwell et al. (1991), however, no dystrophic calcification of necrotic muscle fibres was described in any of their 31 cases despite three to four weeks spent in intensive care. Large deposits of calcium are rarely visible in human muscle biopsies, although they are often seen in the genetic dystrophy of hamsters (Carpenter et al., 2001b). Calcification of necrotic muscle fibres has been described in a HIV-positive patient with rhabdomyolysis (Carpenter et al., 2001b) whilst calcification of the connective tissue of muscle, called calcinosis, occurs in patients

with juvenile dermatomyositis (Carpenter et al., 2001b). However none of these conditions explains the calcification seen in the diaphragm of this child.

CONCLUSION

Histological examination of the diaphragm is not part of many autopsy protocols. We describe three cases where pathology in the diaphragm may have led to death. In the first two cases a predominantly diaphragmatic myositis was either the direct or underlying cause of death. In the third case, calcification of diaphragmatic muscle fibres may have been a reflection of underlying diaphragmatic disease, leading to the initial respiratory arrest and subsequent impaired respiratory function. Had the diaphragm not been sampled for histology during autopsy, the cause of death would have remained unascertained in these cases. In Cases 2 and 3 the clinical history would have raised the question of imposed upper airways obstruction, had the diaphragmatic pathology not been identified, which may, in turn, have resulted in unjustified and unnecessary investigation of the parents and even prosecution. This underscores the need for full histology at postmortem, including diaphragmatic sampling, in all cases of unexplained infant, child and adult deaths.

ACKNOWLEDGEMENTS

We would like to acknowledge the advice given by Professor. C. Sewry, Imperial College London, Dr. M. P. Carey and Dr. P. C. Barber, University of Birmingham, in the preparation of this report. We are also grateful for the technical help provided by the histopathology laboratory, Birmingham Women's Hospital.

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