ORIGINAL ARTICLE

The usefulness of bone marrow aspiration in the diagnosis of Niemann–Pick disease type C in infantile liver disease

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Background: Niemann–Pick disease type C (NPC) is a fatal, autosomal recessive lysosomal storage disease which may present in infancy with cholestatic jaundice and/or hepatosplenomegaly. In cholestatic patients with splenomegaly, a bone marrow aspirate has been advocated as a relatively accessible tissue to demonstrate storage phenomena. Typically in patients with NPC, macrophages with abnormal cholesterol storage, so called foam cells, can be detected in the bone marrow.
Aim: To review our experience of bone marrow aspiration in children with NPC presenting with infantile liver disease.
Methods: A retrospective analysis of 11 consecutive children (8 males) from Birmingham Children's Hospital with NPC presenting with infantile liver disease was undertaken. The diagnosis of NPC was confirmed in all cases by demonstrating undetectable or low rates of cholesterol esterification and positive filipin staining for free cholesterol in cultured fibroblasts.
Results: The median age at presentation was 1.5 months (range 0.5–10). Bone marrow aspirates showed storage cells in only 7/11 cases. Bone marrow aspirates which had storage cells were undertaken at a median age of 11 months while those with no storage cells were undertaken at median age 2.3 months. The overall sensitivity of bone marrow aspirates for detecting storage cells in children presenting with infantile liver disease was 64%; however, for children who had bone marrow aspirates in the first year of life it was only 57%.
Conclusions: The sensitivity of bone marrow aspirate for the diagnosis of NPC disease in patients presenting with infantile liver disease was lower than previously reported. Where NPC is suspected clinically, definitive investigations should be undertaken promptly. There is a need to develop sensitive screening methods for NPC in children presenting with infantile liver disease.

Niemann–Pick disease type C (NPC) is a fatal, autosomal recessive, lysosomal storage disorder characterised by a variable phenotype that includes hepatosplenomegaly, liver dysfunction, progressive neurodegeneration, and a vertical supraopthalmoplegia.[1] These occur secondary to lysosomal accumulation of unesterified cholesterol; the disease is caused by mutations in one of two underlying genes, NPC1 or NPC2.[2 3]

Between 45% and 65% of NPC cases present with neonatal liver disease[4–6] manifest as conjugated hyperbilirubinaemia and/or hepatosplenomegaly. Cholestasis resolves spontaneously by 2–4 months in the majority of infants, but in about 10% of cases it may lead to rapidly progressive liver failure.[7]

Diagnosing NPC is difficult in these infants as the differential diagnosis is wide and neurological features will not have developed. NPC may mimic conditions such as biliary atresia and it is important to have an accurate, quick, and reliable test to help reach a diagnosis. This is important to prevent unnecessary investigations as well as to guide prognosis, genetic counselling, and antenatal diagnosis.[8] Furthermore, in those infants who develop liver failure, an early diagnosis may help avoid unnecessary liver transplantation.[9 10]

The definitive method for diagnosing NPC is the demonstration of undetectable or low rates of cholesterol esterification accompanied by excess storage of free cholesterol by filipin staining in cultured fibroblasts[11–13] or by the detection of two pathogenic mutations.[2 3] These tests are expensive, available only at a few specialised centres and may take a long time for results to become available. This makes them impractical as first line/screening investigations. Early diagnosis rests on maintaining a high level of clinical suspicion and demonstrating characteristic storage cells, usually in liver and/or bone marrow tissue.

Liver histology alone is insufficient for this purpose, as histological features diagnostic of NPC are found on liver biopsy in only 50% of cases, even when the diagnosis is already known.[5] Bone marrow aspiration has been shown retrospectively to be a sensitive and relatively non-invasive method for demonstrating storage material in children with confirmed NPC.[5] As a result, bone marrow aspiration has been recommended in the investigation of unexplained neonatal cholestasis, especially if accompanied by persistent splenomegaly.[5 6] There is however little information on the diagnostic yield of bone marrow aspiration when it is undertaken at the time of presentation with neonatal liver disease.

The aim of this study was to review our experience of bone marrow aspirates in infants with NPC presenting with infantile liver disease.

SUBJECTS AND METHODS

A retrospective review of children proven to have NPC who presented with infantile liver disease between 1985 and 2004 was undertaken. During this time approximately 750 cases of infantile liver disease were assessed at the Liver Unit at Birmingham Children's Hospital. Children were investigated using a structured protocol which evolved throughout the time of this study.[7] In addition to excluding recognised

surgical, metabolic, and infective causes of neonatal liver disease, percutaneous liver biopsy was undertaken in the great majority of cases. Bone marrow aspiration was a discretionary second line investigation indicated where there was unexplained splenomegaly or where no cause of cholestasis had been discovered after first line investigations. All children investigated for infantile liver disease were followed up clinically until a specific diagnosis was established or until they had made a complete clinical and biochemical recovery.

The diagnosis of NPC was established in all cases by demonstrating undetectable or low rates of cholesterol esterification accompanied by demonstrating excess storage of free cholesterol by filipin staining in cultured fibroblasts using published methods.[13]

Data pertaining to their clinical and biochemical status at the time of initial presentation with infantile liver disease, bone marrow biopsy reports with their timings, and the age at which the diagnosis of NPC was confirmed were obtained. A haematologist re-examined all the bone marrow aspirate samples, without knowledge of the original report. The liver biopsies were also re-examined by a histopathologist and these current reports were used for the analysis.

Liver biopsy specimens were fixed in formalin and processed into paraffin wax for routine sections, stained with haematoxylin and eosin, and routine special stains including PAS/PASD. A liver biopsy was regarded as diagnostic for Niemann–Pick disease where foamy macrophages were visible on light microscopy highlighted in relief on PAS staining. Liver biopsy specimens were also preserved in glutaraldehyde for electron microscopy. Bone marrow aspirates were stained with Wright's stain and examined by light microscopy.

RESULTS

Results are presented in table 1. Eleven children (eight male) were identified, accounting for approximately 1.5% of cases of infantile liver disease seen at our centre. The median age at initial presentation was 1.5 months (range 0.5–10). Seven children presented with conjugated hyperbilirubinaemia and hepatosplenomegaly and four had only hepatosplenomegaly. Their median bilirubin level at presentation was 125 μmol/l (range 5–192). The median age at eventual diagnosis of NPC was 10 months (range 1.5–47).

Bone marrow aspirates were done at a median age of 10 months (range 1.5–47). In seven cases the bone marrow aspirate was undertaken at the time of initial evaluation of infantile liver disease. In the remainder this was carried out as a delayed second line investigation where clinical concern persisted.

Seven children (median age at biopsy 11 months; range 1.5–47) had characteristic storage cells found, while four children (median age at biopsy 2.3 months; range 1.5–16) had normal bone marrow aspirates. The bone marrow aspirates were re-examined for the purpose of this study. All samples were found to be of diagnostic quality with adequate cellularity. The original findings were confirmed in all cases with no ambiguous cases discovered.

The overall sensitivity of bone marrow aspirates for detecting storage cells in children presenting with infantile liver disease was 7/11 (64%); however for children who had their bone marrow aspirates in the first year of life it was only 4/7 (57%).

Fourteen liver biopsies were carried out on 10 children. One child presenting at 9 months with hepatosplenomegaly had a diagnostic bone marrow aspirate and a liver biopsy was not undertaken. Nine children had liver biopsies done at the time of their initial presentation at a median age of 1.5 months (range 1.5–11). In only one child was this diagnostic of Niemann–Pick disease (at 11 months of age). The other eight biopsies showed a non-specific pattern typical of idiopathic neonatal hepatitis, with in addition evidence of bile duct paucity in four. In seven cases electron microscopy was undertaken, of which five had abnormal findings. These consisted of abundant myelin figures, which were suggestive but not diagnostic for Niemann–Pick disease. One child who initially presented at 2 months of age did not have invasive investigations in infancy. He subsequently underwent liver biopsy at 29 months because of persistent hepatosplenomegaly. This showed classical diagnostic features of Niemann–Pick disease.

Four children had repeat liver biopsies at a median age of 16.5 months (range 5.5–24) because of persisting unexplained splenomegaly. Three of these now showed diagnostic features of Niemann–Pick disease; one biopsy was of insufficient quality to allow meaningful interpretation. The sensitivity of liver histology for detecting diagnostic features of NPC in children presenting with infantile liver disease was 5/14 (36%); however where the liver biopsies were undertaken in the first year of life it was only 2/10 (20%).

DISCUSSION

In our centre, bone marrow aspirates have proved to be a poor screening test for NPC presenting with infantile liver disease with a sensitivity of only 57% for those done in the first year of life. Strong clinical suspicion remained crucial in establishing the diagnosis and initiating definitive investigations.

Previous studies and correspondence have suggested that false negative bone marrow aspirates for NPC are unusual.[5 14]

Table 1 Timing and results of biopsies in infants presenting with infantile liver disease

Patient	Age at bone marrow biopsy (mth)	Bone marrow aspirate result LM	Age at initial liver biopsy (mth)	Liver biopsy result			Age at repeat liver biopsy (mth)	Liver biopsy result LM
				LM	EM	PBD		
1	1.5	−	1.5	−	s	yes	5.5	+
2	1.5	−	1.5	−	s	no	x	x
3	1.5	+	1.5	−	s	yes	x	x
4	3	+	3	−	s	no	x	x
5	4	−	4	−	s	no	x	x
6	10	+	x	x	x	x	x	x
7	11	+	11	+	x	no	x	x
8	16	−	1.5	−	n	yes	16	+
9	17	+	4	−	x	no	17	+
10	29	+	29	+	x	x	x	x
11	47	+	1.5	−	n	yes	24	poor quality

Details of patients listed according to their age at the time of bone marrow aspirate.
+, presence of storage cells; −, no storage cells seen; s, suggestive; n, non-suggestive; x, not done.
LM, light microscopy; EM, electron microscopy; PBD, paucity of bile ducts.

This is at variance with our experience. Sampling or interpretive errors appear unlikely given that the bone marrow aspirate slides were reviewed by an experienced haematologist, who confirmed the diagnostic quality, adequacy of cellularity, and the original findings of all the samples.

A major strength of the current study is that a clearly defined population was assessed prospectively using a structured protocol with a high rate of clinical follow up. Therefore the chance of further missed cases was low. In contrast, previous studies have been retrospective, based on children in whom the diagnosis of NPC disease was already established, often on the basis of demonstrating storage material.[5] We have found that storage cells are more likely to be found if the bone marrow aspirate was performed later. So, while a positive bone marrow aspirate is specific for NPC, marrow storage cells in NPC may only become apparent as the disease evolves.[15] If this is so, relying solely on early bone marrow aspiration for diagnosis of NPC may be intrinsically flawed. Unfortunately our findings cannot suggest an obvious cut-off age above which bone marrow aspirate is more likely to be diagnostic.

The overall sensitivity of liver biopsies for detecting storage cells in these children was 36%, which is comparable to earlier studies.[5] Our findings suggest that liver histology findings in NPC also develop with time. The earliest liver biopsy that revealed the typical light microscopic features of Niemann–Pick disease was obtained at 5.5 months. Thus, early biopsies may also miss the diagnosis. This is a similar to what we have observed with bone marrow aspirates, and is consistent with previous reports of liver histology in NPC.[16]

Electron microscopy was suggestive of Niemann–Pick disease even before characteristic changes were seen in the corresponding light microscopy results. Although myelin figures can be a non-specific finding in neonatal hepatitis, if they are present in large numbers and not confused with whorled structures seen in bile whenever there is cholestasis, they can be a helpful marker for targeting further investigations.

Interestingly, 4/5 patients biopsied before 2 months of age showed paucity of bile ducts; this resolved in two children who had follow up biopsies. Non-syndromic bile duct paucity is a non-specific finding and can occur from a variety of infective, metabolic, and genetic causes.[17–20] This has only been reported in one previous case of NPC and may be more common than previously suspected. The mechanism of bile duct paucity in NPC is unclear, but may be related to the production of abnormal bile acids which have been reported in NPC.[21] Whether these develop due to unusual metabolism of cholesterol as a consequence of its abnormal subcellular distribution or as a reflection of involvement of NPC1 in the conversion of cholesterol to bile acids is unknown. Our retrospective study cannot shed any light on this matter but it does emphasise the importance of considering NPC in the differential diagnosis of bile duct hypoplasia associated with raised γ-glutamyl transferase.

Our findings highlight the need for a reliable screening test for NPC in children with infantile liver disease to determine who will require definitive investigations. Unfortunately there is no proven method. Serum or plasma chitotriosidase, an endo-β-glucosaminidase secreted by activated macrophages, has been shown to be moderately elevated[22 23] in proven cases of NPC. This test is relatively easy and may be useful as a screening tool, although it has not yet been evaluated for this purpose.

In conclusion, bone marrow aspirates are not reliable as a screening test in the diagnosis of NPC presenting with infantile liver disease. While a positive result is a useful pointer to NPC, a negative result cannot exclude the diagnosis. In children with a high index of clinical suspicion, i.e. especially those presenting with cholestasis and unexplained splenomegaly, skin biopsy for fibroblast culture should be undertaken without delay to enable definitive tests to be performed. Reliable screening tests for NPC are needed.

What is already known on this topic

- Diagnosing NPC in infants presenting with liver disease can be difficult and a high level of clinical suspicion is necessary
- The definitive diagnostic method is to demonstrate abnormal cholesterol esterification in cultured fibroblasts or to detect pathogenic mutations

What this study adds

- Bone marrow aspirates are not reliable as a screening test for NPC presenting with infantile liver disease
- In children with a high index of clinical suspicion, definitive tests (skin biopsy for fibroblasts culture) should be undertaken without delay

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IMAGES IN PAEDIATRICS

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Extreme subcutaneous emphysema of eyelids

We describe a previously healthy 14 month old girl with a one day history of bilateral periorbital swelling. During the previous evening, parents had noticed slight swelling over the neck and right eye, which during the night worsened and spread to the left eye and chest. There was no history of fever, cough, breathing difficulty, trauma, or surgery; however, she had been constipated for the last four days. On examination she was in respiratory distress with gross swelling of the face and eyelids (fig 1). Her temperature was 100°F, pulse 130, and respiratory rate 65 per minute. Crackles were palpable over the neck and chest; *x* ray examination showed pneumomediastinum, subcutaneous emphysema of chest, neck, and orbits (fig 2). She was treated conservatively; the swelling subsided considerably during the next 10 days and disappeared completely after six weeks (fig 3).

Orbital emphysema is an uncommon complication of facial injuries or operative procedures.[1 2] It can also be caused by straining such as the Valsalva manoeuvre or even blowing the nose.[3] Since there was a history of constipation in our patient, it was assumed that straining led to interstitial and subsequently orbital emphysema. Emphysema usually resolves spontaneously in 2–3 weeks, therefore no active treatment is needed, even when it is severe.[4]

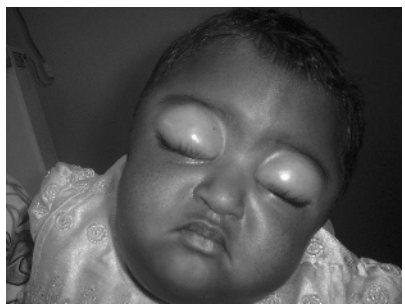

Figure 1 The patient had extreme swelling of the eyelids; the eyes were shut tightly, and the face swollen.

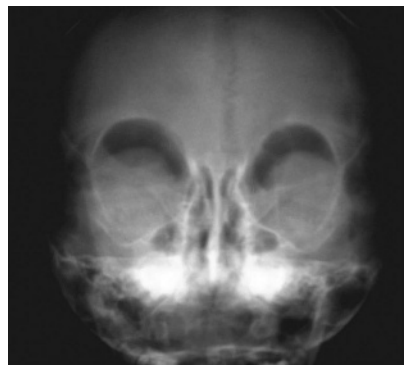

Figure 2 Skull *x* ray showing subcutaneous emphysema of the orbits.

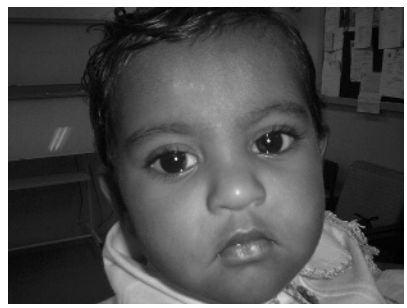

Figure 3 Normal face of the child without swelling of eyelids and face.

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