Introduction

Cryptosporidium is a common protozoan parasitic cause of diarrhoea in children worldwide. In those with profound T-cell immunodeficiency, including haematopoietic stem cell transplant (HSCT) recipients, it can cause protracted disease which may be fatal[1,2]. Its role in sclerosing cholangitis in patients with dedicator of cytokinesis 8 (DOCK 8) deficiency has been highlighted[3]. Specific treatment options are limited, with no licensed treatment in the EU; in the US, treatment with nitazoxanide is licensed for immunocompetent patients. There is no evidence for its efficacy in immunocompromised patients[4]. Some young children display asymptomatic *Cryptosporidium* carriage[5,6] which may precede symptomatic disease in vulnerable groups[2]. Detecting asymptomatic carriage and some symptomatic cases may require more sensitive methods than microscopy of stained smears, such as PCR, immunofluorescence microscopy (IFM), or immuno-magnetic separation (IMS)-IFM[2,6,7] , methods used by very few carriage studies.

This report describes a prospective cohort study of children with primary immunodeficiencies undergoing HSCT in the UK. The study objectives were to use highly sensitive methods to investigate the extent of carriage of *Cryptosporidium* and its clinical significance in this high-risk group of patients.

Methods

Over a two-and-a-half year period, all children <18 years old with primary immunodeficiencies undergoing HSCT at the paediatric bone-marrow transplant (BMT) units at the Royal Victoria Infirmary, Newcastle-upon-Tyne and Great Ormond Street Children's Hospital, London were eligible for inclusion in the study. Between them, these two centres perform the vast majority of BMTs in this patient group for the UK and Ireland.

Informed consent to participate in the study was obtained from patients and/or their guardians. Clinical patient data was supplied by the clinical team caring for the patients by means of a structured questionnaire. Possible risk factors for exposure were obtained from families, who filled in a questionnaire which asked about the following risk factors: travel history, number of children living in same household, water supply at home (mains supply, private supply or group water scheme if in Ireland), whether drinking water had been boiled, swimming, pets, farm visits, nature and duration of childcare and school attendance. Stools from all study participants were collected prior to transplant and tested by routine microscopy (with modified Ziehl-Neelsen or Auramine phenol staining) in the local diagnostic laboratory and then in all cases by specialist tests at the national *Cryptosporidium* Reference Unit as follows: IFM (Crypto-Cel, Cellabs); PCR (SSU rRNA gene)[8]; IMS-IFM (Isolate, TCS Biosciences; Crypto-Cel, Cellabs)[7]. In stools found to be *Cryptosporidium*-positive the species and subtype was confirmed by sequencing PCR products amplified from the SSU rRNA and *gp60* genes[8]. Repeat samples were tested at 2 months post-transplant, and again at 3 months after the end of immunosuppression (to give the patient a chance to clear carriage). Specimens were also tested on clinical grounds whenever a patient had symptoms consistent with cryptosporidiosis. Clinical and patient follow-up data were collected.

Results

Forty-two patients undergoing BMT for primary immune deficiency were recruited: 34 from the UK, 7 from the Republic of Ireland and one from Norway. The age range was 1 month to 17 years; median 2.5 years, mean 7.4 years (10 children aged <1 year, 8 children aged 1-2 years, 8 children aged 2-5 years, 16 children aged 7-17 years). The underlying diagnoses were: Severe Combined Immune Deficiency (SCID) (8 children), Chronic Granulomatous Disease (7), CD40 ligand deficiency (5), Hemophagocytic lymphohistiocytosis (3), DOCK 8 deficiency (2), combined immunodeficiency syndrome (2), Omenn's syndrome (2), immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)-like syndrome (2), one with each of Cartilage Hair Hypoplasia, X-linked lymphoproliferative (XLP)-like syndrome, immunodeficiency, centromeric region instability, and facial anomalies (ICF) syndrome, Fas-associated death domain protein (FADD) deficiency, osteopetrosis, Wiskott-Aldrich syndrome, 2 with complex autoimmune disease, 3 unclassified.

Three patients were found to be infected with *Cryptosporidium*. The presentation and clinical impact of the disease in these three cases were very different from one another. One patient (case 1) was infected with *Cryptosporidium parvum* (subtype IIaA19G4R1). This case was a 17 year old male from Ireland who had first presented at the age of 5 years. The presentation and course of his undefined combined immunodeficiency resembled CD40 Ligand deficiency although this was excluded. At the age of 8 years he had developed hepatosplenomegaly, diarrhoea and cholangiohepatitis. At that time his stools were consistently negative for *Cryptosporidium* by microscopy at his local microbiology laboratory. However a liver biopsy revealed histological evidence of *Cryptosporidium* and advanced liver disease.

Eventually three years later *C. parvum* (subtype IIaA19G4R1) was detected in a small bowel aspirate and subsequently was detectable intermittently in stool samples. He suffered severe disease attributable to the infection, leading to cholangitis and liver cirrhosis. At the age of 14 years he underwent a liver transplant. Six weeks later HSCT was performed. His first liver was rejected but a second transplant at age 15 was successful. He was treated with nitazoxanide and azithromycin throughout (unlicensed indications). His stools remained positive for *Cryptosporidium* a few months after his second liver transplant and he continued on nitazoxanide for almost two years after that, in view of his immunosuppressant treatment, and concern about the new liver becoming infected. Long term

azithromycin was continued as part of his routine post-HSCT antibacterial prophylaxis. He had a number of risk factors for *Cryptosporidium* infection, most notably drinking unboiled water from the household private water supply, and living on a farm where he came into direct contact with cows and sheep.

Two cases had *Cryptosporidium hominis* but, surprisingly, did not appear to suffer clinical disease. One (case 2) was an 11 year old girl from the UK and the other (case 3) a 7 year old boy from Ireland, both with DOCK 8 deficiency. In both, stool screening by microscopy pre-transplant was negative. In case 2, stool screening was positive for *C. hominis* at seven weeks post–HSCT when her CD4 count was 102 cells/mm^3, but the patient was asymptomatic. She was nonetheless treated with azithromycin and nitazoxanide for 1 week (unlicensed indication). Treatment was then discontinued and the patient's CD4 counts rose to 586 cells/mm^3 within two more weeks. Her risk factors for *C. hominis* included contact with three younger siblings and infrequent use of swimming pools. She drank unboiled tap water from a mains supply. Four weeks after the first positive sample, stool microscopy was negative although still positive for *Cryptosporidium* by PCR. In case 3, routine screening locally by microscopy revealed presence of *Cryptosporidium* (identified as *C. hominis*) four weeks post-transplant when CD4 count was 387 cells/mm^3. This boy was also asymptomatic. He was treated for one week with azithromycin (unlicensed indication). CD4 count reached 479 cells/mm^3 two weeks later. Stool remained positive for *Cryptosporidium* by both microscopy and PCR for eight weeks after infection but became negative after ten and fifteen weeks respectively. He had been in the UK for four months, during which time he drank only boiled/filtered water. Risk factors for *C. hominis* included one younger sibling, using swimming pools (though not in the year prior to stool sampling), and attendance at day nursery and childminder for two years before starting school.

Typically one would expect to find more severe disease in this vulnerable group but the identification of these cases may indicate that asymptomatic carriage is more common than currently believed, and is perhaps under-detected. These two cases presented within one month of each other in the same transplant unit. However, different gp60 subtypes were identified: IbA10G2 and IfA13G1, a finding which did not support the occurrence of transmission between these two patients within the unit. Increased observation and testing of the 9 patients on the unit at that time detected no further *Cryptosporidium* cases either clinically or microbiologically by testing stools using sensitive methods.

Discussion

Three of the cases (3/42; 7%) were found to be infected with *Cryptosporidium*, more than 5 times the proportion detected using the same techniques among healthy children in a UK study of young children attending day-care settings[6]. All three cases occurred in children in the older age range (8, 11 and 7 years at first presentation). In developed countries, infants and children aged less than 2 years may be less likely to have been exposed to *Cryptosporidium,* particularly if they have presented with immune deficiency at a very young age and provided with precautionary advice. If cases aged <2 are excluded from the analysis, 3/24 (12.5%) were infected.

In a previous study by Mclauchlin et al[2], 12 of 25 (48%) children with primary immunodeficiencies tested prospectively were reported positive by PCR (but not microscopy) for *Cryptosporidium* – a much higher proportion than found in our study. They were of a relatively older age group than our series but nonetheless the proportion infected was about four times that in our cohort even after excluding the under-twos. Mclauchlin's cases were studied 10-15 years prior to our study when UK drinking water supply quality was not as good, and there was lower awareness of the risk of *Cryptosporidium* to this patient group. Since that time, the Water Supply (Water Quality) Regulations of 2000 were introduced and an associated decline in cryptosporidiosis has been demonstrated[9]. Additionally, these high risk patients have been managed with strict advice on avoiding *Cryptosporidium*[10]. In Mclauchlin's cohort the children became sicker during transplant as a result of cryptosporidiosis. This might at least partly be explained by changes in the intensity of chemotherapy conditioning. However the underlying diagnosis may also be relevant. In McLaughlin's study, nearly half (46%) had CD40 ligand deficiency; in ours it was only 5/42 (12%). Of our three cases, the case resembling CD40 ligand deficiency was most severely affected. The worst affected child also had *C. parvum* infection whilst the other two were infected with *C. hominis,* although the numbers in this study are too small to draw any conclusion regarding prognosis by infecting species of *Cryptosporidium*.

Whilst overall only 1/34 of study patients from the UK were infected with *Cryptosporidium*, 2/7 of those from Ireland were affected. The numbers in this study are small, however a study including more patients would be lengthy, since given the rarity of these conditions our patients took two years to recruit. Regulations

supporting the European Drinking Water Directive and water safety plan approach are now being implemented in both countries.

Conclusions

This study provides an indication of the current frequency and presentation of cryptosporidiosis within this patient group, and of geographical issues to consider as to a patient's origin during initial assessment. Screening may be justified for patients from some locations; however specialist pre-HSCT stool screening did not result in any change in patient management in this series. Although patients are at risk of infection post-transplant, lower intensity conditioning may have limited the clinical significance provided the immune system is recovering.

Acknowledgements

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Ethical Approval

This study was carried out with the ethical approval of the relevant UK NHS Research Ethics Committee and all required NHS R&D permissions. Informed consent was obtained from all individual participants included in the study and/or their guardians.

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