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1 **Introduction**

2 *Cryptosporidium* is a common protozoan parasitic cause of diarrhoea in children
3 worldwide. In those with profound T-cell immunodeficiency, including haematopoietic
4 stem cell transplant (HSCT) recipients, it can cause protracted disease which may be
5 fatal^{1,2}. Its role in sclerosing cholangitis in patients with dedicator of cytokinesis 8
6 (DOCK 8) deficiency has been highlighted³. Specific treatment options are limited,
7 with no licensed treatment in the EU; in the US, treatment with nitazoxanide is
8 licensed for immunocompetent patients. There is no evidence for its efficacy in
9 immunocompromised patients⁴. Some young children display asymptomatic
10 *Cryptosporidium* carriage^{5,6} which may precede symptomatic disease in vulnerable
11 groups². Detecting asymptomatic carriage and some symptomatic cases may require
12 more sensitive methods than microscopy of stained smears, such as PCR,
13 immunofluorescence microscopy (IFM), or immuno-magnetic separation (IMS)-
14 IFM^{2,6,7}, methods used by very few carriage studies.

15

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

20

21 **Methods**

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

28

29 Informed consent to participate in the study was obtained from patients and/or their
30 guardians. Clinical patient data was supplied by the clinical team caring for the
31 patients by means of a structured questionnaire. Possible risk factors for exposure
32 were obtained from families, who filled in a questionnaire which asked about the
33 following risk factors: travel history, number of children living in same household,
34 water supply at home (mains supply, private supply or group water scheme if in
35 Ireland), whether drinking water had been boiled, swimming, pets, farm visits, nature
36 and duration of childcare and school attendance. Stools from all study participants
37 were collected prior to transplant and tested by routine microscopy (with modified
38 Ziehl-Neelsen or Auramine phenol staining) in the local diagnostic laboratory and
39 then in all cases by specialist tests at the national *Cryptosporidium* Reference Unit as
40 follows: IFM (Crypto-Cel, Cellabs); PCR (SSU rRNA gene)⁸; IMS-IFM (Isolate, TCS
41 Biosciences; Crypto-Cel, Cellabs)⁷. In stools found to be *Cryptosporidium*-positive
42 the species and subtype was confirmed by sequencing PCR products amplified from
43 the SSU rRNA and *gp60* genes⁸. Repeat samples were tested at 2 months post-
44 transplant, and again at 3 months after the end of immunosuppression (to give the
45 patient a chance to clear carriage). Specimens were also tested on clinical grounds
46 whenever a patient had symptoms consistent with cryptosporidiosis. Clinical and
47 patient follow-up data were collected.

48

49 **Results**

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

63
64 Three patients were found to be infected with *Cryptosporidium*. The presentation and
65 clinical impact of the disease in these three cases were very different from one
66 another. One patient (case 1) was infected with *Cryptosporidium parvum* (subtype
67 IlaA19G4R1). This case was a 17 year old male from Ireland who had first presented
68 at the age of 5 years. The presentation and course of his undefined combined
69 immunodeficiency resembled CD40 Ligand deficiency although this was excluded. At
70 the age of 8 years he had developed hepatosplenomegaly, diarrhoea and
71 cholangiohepatitis. At that time his stools were consistently negative for
72 *Cryptosporidium* by microscopy at his local microbiology laboratory. However a liver
73 biopsy revealed histological evidence of *Cryptosporidium* and advanced liver
74 disease.

75
76 Eventually three years later *C. parvum* (subtype IlaA19G4R1) was detected in a
77 small bowel aspirate and subsequently was detectable intermittently in stool
78 samples. He suffered severe disease attributable to the infection, leading to
79 cholangitis and liver cirrhosis. At the age of 14 years he underwent a liver transplant.
80 Six weeks later HSCT was performed. His first liver was rejected but a second
81 transplant at age 15 was successful. He was treated with nitazoxanide and
82 azithromycin throughout (unlicensed indications). His stools remained positive for
83 *Cryptosporidium* a few months after his second liver transplant and he continued on
84 nitazoxanide for almost two years after that, in view of his immunosuppressant
85 treatment, and concern about the new liver becoming infected. Long term

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

90

91 Two cases had *Cryptosporidium hominis* but, surprisingly, did not appear to suffer
92 clinical disease. One (case 2) was an 11 year old girl from the UK and the other
93 (case 3) a 7 year old boy from Ireland, both with DOCK 8 deficiency. In both, stool
94 screening by microscopy pre-transplant was negative. In case 2, stool screening was
95 positive for *C. hominis* at seven weeks post-HSCT when her CD4 count was 102
96 cells/mm³, but the patient was asymptomatic. She was nonetheless treated with
97 azithromycin and nitazoxanide for 1 week (unlicensed indication). Treatment was
98 then discontinued and the patient's CD4 counts rose to 586 cells/mm³ within two
99 more weeks. Her risk factors for *C. hominis* included contact with three younger
100 siblings and infrequent use of swimming pools. She drank unboiled tap water from a
101 mains supply. Four weeks after the first positive sample, stool microscopy was
102 negative although still positive for *Cryptosporidium* by PCR. In case 3, routine
103 screening locally by microscopy revealed presence of *Cryptosporidium* (identified as
104 *C. hominis*) four weeks post-transplant when CD4 count was 387 cells/mm³. This boy
105 was also asymptomatic. He was treated for one week with azithromycin (unlicensed
106 indication). CD4 count reached 479 cells/mm³ two weeks later. Stool remained
107 positive for *Cryptosporidium* by both microscopy and PCR for eight weeks after
108 infection but became negative after ten and fifteen weeks respectively. He had been
109 in the UK for four months, during which time he drank only boiled/filtered water. Risk
110 factors for *C. hominis* included one younger sibling, using swimming pools (though
111 not in the year prior to stool sampling), and attendance at day nursery and
112 childminder for two years before starting school.

113

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

123

124

125 **Discussion**

126 Three of the cases (3/42; 7%) were found to be infected with *Cryptosporidium*, more
127 than 5 times the proportion detected using the same techniques among healthy
128 children in a UK study of young children attending day-care settings⁶. All three cases
129 occurred in children in the older age range (8, 11 and 7 years at first presentation). In
130 developed countries, infants and children aged less than 2 years may be less likely to
131 have been exposed to *Cryptosporidium*, particularly if they have presented with
132 immune deficiency at a very young age and provided with precautionary advice. If
133 cases aged <2 are excluded from the analysis, 3/24 (12.5%) were infected.

134

135 In a previous study by Mclauchlin et al², 12 of 25 (48%) children with primary
136 immunodeficiencies tested prospectively were reported positive by PCR (but not
137 microscopy) for *Cryptosporidium* – a much higher proportion than found in our study.
138 They were of a relatively older age group than our series but nonetheless the
139 proportion infected was about four times that in our cohort even after excluding the
140 under-tuos. Mclauchlin's cases were studied 10-15 years prior to our study when UK
141 drinking water supply quality was not as good, and there was lower awareness of the
142 risk of *Cryptosporidium* to this patient group. Since that time, the Water Supply
143 (Water Quality) Regulations of 2000 were introduced and an associated decline in
144 cryptosporidiosis has been demonstrated⁹. Additionally, these high risk patients have
145 been managed with strict advice on avoiding *Cryptosporidium*¹⁰. In Mclauchlin's
146 cohort the children became sicker during transplant as a result of cryptosporidiosis.
147 This might at least partly be explained by changes in the intensity of chemotherapy
148 conditioning. However the underlying diagnosis may also be relevant. In
149 McLaughlin's study, nearly half (46%) had CD40 ligand deficiency; in ours it was only
150 5/42 (12%). Of our three cases, the case resembling CD40 ligand deficiency was
151 most severely affected. The worst affected child also had *C. parvum* infection whilst
152 the other two were infected with *C. hominis*, although the numbers in this study are
153 too small to draw any conclusion regarding prognosis by infecting species of
154 *Cryptosporidium*.

155

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

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

162

163 **Conclusions**

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

171

172 **Acknowledgements**

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174

175 **Ethical Approval**

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

180

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