**The detection of rotavirus in paediatric oncology patients with diarrhoea: the impact of rotavirus vaccine.**

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**Summary:**

We reviewed seven year's data to examine stool testing for rotavirus in patents treated on a regional Paediatric Oncology Unit before and after the introduction of UK-wide rotavirus immunisation in July 2013.

We showed that the prevalence of rotavirus positivity has diminished since the introduction of rotavirus immunisation with 21 of 416 positive samples between 2010 and 2012 but only one positive test out of 122 samples in 2015/2016. Based on these results, we suggest there is very little utility for routine rotavirus testing in children and young people with cancer presenting with diarrhoea.

**Background**

Diarrhoea is a frequent problem in paediatric oncology patients. The causes of diarrhoea in children and young people being treated for cancer include infections, antibiotics, chemotherapy, and radiotherapy. Less common aetiologies include graft versus host disease and anxiety related to cancer diagnosis. 1

Diarrhoea leads to complications such as fluid loss, electrolyte imbalance and acute renal failure. It may be fatal if not treated in a timely fashion.

Stool samples can be tested for a variety of infective causes of diarrhoea. These include viruses, parasites and bacteria including toxin-associated *Clostridium difficile* infection.

Before the introduction of rotavirus vaccine, rotavirus was the most common pathogen associated with paediatric diarrhoea. Other less commonly associated viruses include novovirus, enteric adenovirus, astrovirus and sapovirus. 2 3

Rotavirus is a 70 nm, non-enveloped RNA virus. It has a viral genome of double stranded RNA, which is surrounded by a triple layered protein capsid. The VP7 glycoprotein and the VP4 protein are outer layer structural proteins. These two proteins induce the production of neutralising antibodies.

 and determineregulate the G and P serotypes respectively and which have been used in the development of rotavirus vaccines.

Mechanisms responsible for diarrhoea related to rotavirus include loss of epithelium leading to loss of brush border enzymes, effect of rotavirus enterotoxin and activation of the enteric nervous system. The destruction of epithelium leading to loss of enzymes, leads to carbohydrate malabsorption resulting in an osmotic type of diarrhoea. The spread of disease is via faecal-oral route; the incubation period is less than 48 hours. A small inoculum is needed for transmission, typically no more than 100 colony-forming units per gram. 5

Two oral live attenuated rotavirus vaccines are available to be used; RotarixTM, a monovalent vaccine derived from the most common human rotavirus serotype (G1P[8]), and secondly a pentavalent vaccine, RotaTeqTM, incorporating five commonhuman serotypes (G1, G2, G3, G4 and P[8])

The World Health Organisation has recommended rotavirus vaccine in the childhood immunisation schedule globally. It was first licensed in 2006. Vaccination has been added to national vaccination schedule over time in a number of countries; 82 countries had started using it by December 2016. 6 Rotavirus vaccine is extremely effective against gastroenteritis in middle and high-income countries with a considerable positive effect on Public Health in developed countries. The proportion of severe gastroenteritis and mortality related to rotavirus infection is also decreased in low income countries. 7 8 In United Kingdom, rotavirus vaccine was introduced in national vaccination schedule in July 2013 using RotarixTM. 9 A study at our institution of 1644 hospitalized children that tested positive for rotavirus between July 2002 and June 2015 suggested that rotavirus vaccination had rapidly reduced the rotavirus disease burden for both community and hospital acquired cases. 10

There are very few published studies that have evaluated the benefit of testing for common infective agents in paediatric oncology patients with diarrhoea. One such study was performed in our own center that assessed the yield of routine stool microscopy and culture. This study revealed that less than 0.1 percent stool tests were positive for bacterial pathogens and as a consequence we have discontinued the routine testing of stool for bacterial pathogens in children and young people with cancer presenting with diarrhoea. 1

Following on from this study, we undertook a service evaluation to assess the results of stool sent for enteric virus testing before and after rotavirus vaccine introduction. This study focused on rotavirus alone as this was routinely tested over 20 years whereas the policy for testing for other viruses varied over time.

 To determine the practice of routine rotavirus testing the UK, we sent the questionnaire to other Paediatric Oncology Primary Treatment Centers in the UK and the Republic of Ireland (Childrens Cancer and Leukaemia [CCLG] affiliated centres).

**Material and methods**

Setting

This retrospective, descriptive study was conducted at Alder Hey Children’s Hospital, which provides primary, secondary, and tertiary care facilities for more than 270,000 children each year. It has 270 inpatient beds. The oncology department treats around 130 new referrals each year. The Haematology / Oncology Unit has a large catchment area for patients from Merseyside and the North West to the Isle of Man, North Wales, and up as far as parts of Lancashire and Cheshire.

Study samples and participants

The **ProSpecT™** enzyme linked immunosorbent assay was used for the qualitative detection of rotavirus antigen in stool. We examined the results of the stool samples sent for routine rotavirus analysis from the paediatric oncology unit, over a 7 year period, from January 2010 to December 2016. During this time it was routine practice to send stools for rotavirus testing in all paediatric oncology patients with diarrhoea. The patients included those with solid tumours or haematological malignancies presented to day care centre or admitted as inpatient.

This study therefore included stool tests results undertaken approximately three and half years before, and a similar time after, rotavirus vaccine introduction in the UK.

A national survey was sent to other CCLG affiliated centres in the UK and the Republic of Ireland paediatric oncology treatment centres to determine their current practices with respect to virology testing in patients with diarrhoea, using a proforma (Figure 1).

Data Collection

Our laboratory computerised microbiology records were examined retrospectively. Stool samples repeated within two weeks were not included in the analysis, as it was assumed that symptoms were part of the same episode of gastroenteritis.

**Results**

Alder Hey results

A total of 1118 samples were reviewed.  After rejecting repeat samples taken within two weeks, a total of 819 samples formed the basis for the analysis.  Of these 819 samples, 46 (5.6%) were positive for rotavirus from a total of 38 patients (age 0-19 years, 19 males; 19 female).  The results by year (positives/numbers tested and percentages) were as follows: 2010 (15/141,10.6%), 2011 (10/143,6.9%), 2012 (6/132(4.5%), 2013 (8/158, 5.1%), 2014 (5/123,4.1%), 2015 (0/68,<0.1%), 2016(1/54,(1.9%) (Table 1). None of the rotavirus positive patients from 2013 to 2016 had recently received rotavirus vaccine prior to stool testing.

National Survey

All 20 of the CCLG affiliated Primary Treatment Centres other than Alder Hey responded. Twelve centres had unit guidelines or pathway for the management of diarrhoea and 8 did not. Ten centres had guidelines for ordering stool virology testing and 10 did not. Thirteen centres 'always' order stool virology in patients with diarrhoea, one centre ‘very frequently (75-99%), 3 centres 'usually' (50-74 %) and 3 'sometimes' (25-49 %). A few centres reported the use of specific testing during 'outbreaks' with one centre stating that their testing policy was seasonal. The large majority of centres (19/20) tested stools for both rotavirus and adenovirus, with some centres testing for other viruses such as norovirus. One centre routinely testing for six viruses, one testing for five viruses and one centre, three viruses. Two centres reported testing for norovirus during outbreaks but this practice might well apply in most centres.

All but 3 centres stated that their policy did not depend on the patients neutrophil count. Sixteen centres stated there unit had no change in practice based on factors such as stem cell transplant recipients, whereas four units stated that they had a such a variation in policy.

**Discussion**

A comprehensive service evaluation of all stool tests performed for rotavirus performed in our paediatric oncology patients during a 7-year period has demonstrated that the incidence of rotavirus positivity has diminished with a clear decline of rotavirus detection temporally associated with the introduction of rotavirus vaccine. There was only one positive test out of 122 samples in 2015/2016.  Based on these results, we suggest there is very little utility for routine rotavirus testing in children and young people with cancer presenting with diarrhoea.

After a recent service evaluation on routine bacterial stool cultures in our department we no longer send samples for routine stool microscopy and culture, unless there are complicating symptoms in addition to diarrhoea, for example abdominal pain, blood in stool and other significant symptomatology.

As a result of this current study, we now also no longer routinely send for rotavirus testing. In a similar vein to our bacteriological testing policy, we will continue to test stools for rotavirus and other viruses (on discussion with the microbiology department) in prolonged and severe cases of gastroenteritis. In addition, we will continue to test stool for viruses including adenovirus in stem cell transplantation patients with diarrhoea although specific treatment for adenoviral infection is usually guided by the results of blood PCR. Furthermore we will test for appropriate viruses at the time of outbreaks.

This evaluation suggests that the global effectiveness of rotavirus vaccination for childhood diarrhoea extends to children and young people being treated for cancer. Although from our survey, the majority of UK units routinely test for enteric viruses in patients with diarrhoea (both rotavirus and adenovirus) we propose a re-evaluation of such practice. This could address the utility of routine testing for both rotavirus and adenovirus. This study was limited by the fact that only rotavirus results were examined but, as above, this was the only virus consistently tested by our unit over recent years. With or without the use of stool testing we suggest that the policy for isolating oncology patients presenting to a unit with diarrhoea should not depend on the results from stool microbiological testing although it is certainly reasonable to test stools for patients with prolonged diarrhoea or those with clinical important symptoms.

This study was easy to perform and provides further useful information to guide the appropriate use of laboratory facilities in the paediatric oncology population.

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