Gastrointestinal comorbidity, autistic regression and Measles-containing vaccines: positive re-challenge and biological gradient

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Abstract

Background: A temporal association between exposure to measles-containing vaccine (MCV) and autistic-like developmental regression in a subset of children with enterocolitis has been reported. Measles virus (MV) was detected in ileal biopsies from these children at higher prevalence than in developmentally normal pediatric controls.

This study tested the hypothesis of a dose-response effect of MCV exposure on intestinal pathology, as evidence of a causal association.

Methodology/Principle Findings: Children with normal early development and autistic-like developmental regression were divided into two groups: re-exposed children (n=23), who had received more than one dose of a measles-containing vaccine (MCV), and once-exposed children (n=23), who had received only one dose of MCV. The groups were matched for sex, age, and time-elapsed from first exposure to endoscopy. Comparisons included: secondary (2^o) gastrointestinal (GI) and related physical symptoms and observer-blinded scores of endoscopic and histological disease. Re-exposed children scored significantly higher than once-exposed for 2^o physical symptoms including incontinence, presence of severe ileal lymphoid hyperplasia, number of biopsies with epithelial damage and number of children with acute inflammation. Markers of acute inflammation included number of children affected and proportion of biopsies affected

Conclusion/Significance: The data identify a re-challenge effect on symptoms and a biological gradient effect on intestinal pathology, which links MCV exposure to autistic-like developmental regression and enterocolitis.

Keywords: gastrointestinal comorbidity, measles vaccine, enterocolitis, ileal lymphoid hyperplasia, autism spectrum disorder

1. Introduction

Autism spectrum disorders (ASDs) are a complex set of developmental disorders of childhood, characterized by pervasive impairments in social interaction, deficits in verbal and non-verbal communication and stereotyped, repetitive patterns of behavior and interests. Manifestations frequently begin within the first three years of life. The prevalence of ASD diagnoses has increased substantially over the last decade in developed countries [1,2]. There is a growing awareness of gastrointestinal (GI) and immunological co-morbidity in some ASD children [3-6] that, for those with GI symptoms, may be associated with later onset of behavioral deterioration [7].

An ASD phenotype has recently been described that is associated with developmental/behavioral regression, enterocolitis, and immune abnormalities [3-6,8,9]. Parental reports from the U.K., the U.S., and elsewhere frequently cite exposure to the measles-mumps-rubella (MMR) vaccine as the trigger for their child's physical and behavioral deterioration. The characteristic intestinal pathology in the affected children – ileocolonic lymphoid nodular hyperplasia (LNH) and mucosal inflammation – and the systemic immunologic abnormalities are consistent with a viral etiology [10].

Flow cytometric and immuno-histochemical analysis of mucosal lymphocyte populations in ASD children have demonstrated qualitatively consistent abnormalities at different anatomic sites, indicating a relatively homogenous mucosal lymphocyte infiltrate of predominantly $CD8^+$ phenotype [8,9,11]. The cytokine profile of this lymphocyte infiltrate includes a significant increase in the proportion of $CD3^+TNF\alpha^+$ cells and a significant decrease in the proportion of $CD3^+IL\text{-}10^+$ cells compared with non-diseased pediatric controls [12]. This constellation of pathology is reminiscent of HIV enteropathy [13,14].

Recent reports have implicated MV as one possible etiological agent, and indicate the presence of MV antigen [10] and genomic RNA [15] in foci of hyperplastic gut mucosal lymphoid tissue. Singh *et al.* have reported an atypical humoral immune response to MV in ASD children that correlates with abnormal serum antibody titers to myelin basic protein [16,17]. These findings are consistent with, but not proof of, a causal relationship between MV in some form and ASD in a subset of affected children.

Challenge re-challenge refers to a situation where re-exposure of an individual to an agent (e.g., a drug or a toxin) elicits a similar adverse reaction to that seen following the initial exposure. The secondary reaction associated with re-challenge may either reproduce the features associated with the primary challenge, or may lead to worsening of the condition that was provoked or induced by the initial exposure. Alternatively, the risk of an adverse outcome may increase with increasing exposure.

During the course of our clinical investigations, we have observed that some children who received a second dose of MMR or boosting with the combined measles rubella (MR) vaccine experienced further deterioration in their physical and/or behavioral symptoms following re-exposure. It was stated in April 2001 by the Vaccine Safety Committee of the U.S. Institute of Medicine (IOM) that, in the context of MMR vaccine as a possible cause of autism "*challenge re-challenge would constitute strong evidence of an association*" [18].

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This study tested the hypothesis that GI features, including symptoms and mucosal pathology, may exhibit a different pattern and degree of severity in ASD children who received more than one dose of MMR/MR compared with ASD children who received only one dose.

2. Patients and Methods

From the cohort of 179 children with developmental disorders and GI symptoms (ASD children) referred for a gastroenterological opinion to the Royal Free Hampstead NHS Trust, all children were identified, from hospital record review, who had received more than one dose of a measles-containing vaccine (MCV) (monovalent measles, MMR or MR). These constituted the "re-exposed" group. Developmental diagnoses had been made by appropriate specialists prior to referral for GI opinion.

A child was further eligible for inclusion if there was no documented evidence of abnormal development from clinical records for at least the first 12 months of life. All but three children had received a diagnosis of an ASD. The exceptions were one girl who was under review with a provisional diagnosis of a complex learning disorder, one boy who had a diagnosis of attention deficit hyperactivity disorder (ADHD) with autistic features and a complex learning disorder, and one girl who was considered to have a variant of Rett's syndrome. The last child was included since, in common with other affected children, she had ileal LNH and enterocolitis and was positive for MV RNA in intestinal lymphoid tissue and blood. In addition, while the predisposing genetic lesion for Rett's is known, infectious, enteritic and vaccine exposures are reported to be triggers for the development of this syndrome [19,20].

An important consideration in those elements of this study that rely upon reporting of historical symptoms is recall bias: once a possible association between exposure and outcome has been made, subsequent responses may be biased in favor of this association. In addition, it is possible that physical deterioration, with apparently long-term residual effects, is part of the natural history of some ASDs. In an attempt to address these possibilities in the design of this study, a comparison sample was included. For each case, a child was identified who had no evidence of abnormal development in clinical records during at least the first 12 months of life and a formal diagnosis of developmental disorder (all ASD), as described above, but who had received only one MCV ("once-exposed"). Re-exposed and once-exposed ASD children were matched as closely as possible for sex, age, and time elapsed from first MCV exposure to GI endoscopy.

In order to minimize potential selection bias, the once-exposed group was selected consecutively from the list of those ASD children investigated in the pediatric GI clinic.

3. Clinical History

Efforts were made to obtain all routine clinical, developmental and educational records on each child. These included records from general practitioners (including vaccination records), health visitors, outpatient and inpatient attendances and parents. Medical history and age of symptom onset were determined on the basis of the available information. The focus of this study is GI aspects of the children's medical history. An analysis of the developmental history of these children is beyond its scope.

The term "primary physical symptoms" (1^{o} symptoms) refers to the onset of novel GI, or other potentially related physical symptoms (e.g., failure to thrive) after the first MCV (MCV1). The term "secondary physical symptoms" (2^{o} symptoms) refers to the chronic exacerbation of 1^{o} symptoms, or acquisition of new GI and related symptoms, which must, by definition, have followed 1^{o} symptoms. Symptoms were ascertained from the medical records and are based upon the parental history given to the pediatric gastroenterologist at the patient's initial presentation. Data were cross-checked in the follow-up interview with the parents, which was possible for more than 90% of children.

4. Ileocolonoscopy and Histopathology

All children had undergone ileocolonoscopy. Symptomatic indications for ileocolonoscopy included diarrhea (sometimes spurious, in association with fecal impaction), abdominal pain and bloating, and failure to thrive (see below). No children were receiving anti-inflammatory or gastrointestinal medications. All endoscopic and histological data were gathered prospectively as described previously [4], without awareness of the vaccination status of the child. The assessed features included presence and grade of ileal LNH: absent (0), mild (1), moderate (2), and severe (3). The qualitative and quantitative evaluation of ileal LNH in these children has been described previously [4,8]. In addition, the mucosal features of: colonic LNH, red halo sign [21], loss of vascular pattern, granularity, erythema, and ulceration, were scored as present (1) or absent (0), as described previously [4]. Colonic biopsies were obtained from the cecum; the rectum; and the ascending, transverse, and descending/sigmoid colon. All ileal and colonic biopsies were scored on a standard pro-forma as described and validated previously [4]. The same pathologist scored all the biopsies from re-exposed and once-exposed children. All scoring was conducted prospectively, without any knowledge of the vaccination status or specific developmental history of the child. Biopsies were scored for the presence and grade of acute (neutrophilic) and chronic (lymphocytic) inflammation, epithelial and crypt pathology, lymphoid reaction, and eosinophil infiltration. Presence of acute mucosal inflammation, characterized by neutrophil infiltration of the lamina propria, cryptitis, and crypt abscess formation, reflects more severe, active disease. All children were screened for celiac disease by anti-endomyseal antibody testing. Routine stool culture and microscopy, and serological analyses were performed to screen for common pathogens.

5. Statistical Analysis

Data analysis was carried out using SPSS for Windows v11. Analysis comprised cross-tabulation with p values reported on the chi-square statistic. Where the expected count in any cell was less than 5, Fisher's Exact Test was used. The relative risk statistic (RR) is presented as an estimate of the differential risk associated with re-challenge. 95% Confidence Intervals (CIs) provide information about the degree of certainty around the point estimate with an indication of 5% statistical significance.

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A CI which includes the value of 1 indicates a p value of >0.5. Owing to differences in site and number of biopsies per child, and frequency of ileal examination during endoscopy, data were analyzed using unpaired analysis. The Mann-Whitney test was used to compare time elapsed between (i) those with and without acute mucosal inflammation, and (ii) those with grade III (severe) and grade I (mild) ileal LNH. Statistical significance for all relevant analyses was accepted to be $p \leq 0.05$.

6. Ethics Approval

Ileocolonoscopy and histopathology were performed according to clinical need with informed parental consent. Record review was undertaken as part of studies already approved by the Ethical Practices Committee of the Royal Free Hampstead NHS Trust. In order to minimize the potential for reporting and recall bias parents were not made aware of the specific aims of the study, although they were aware of the potential association between exposure to MCV and onset of an ASD.

7. Results

After exclusion of children for whom satisfactory documentation or corroborative evidence was not available, adequate clinical records on 23 re-exposed and 23 once-exposed ASD children were available for comparison. Table 1 provides a summary of the relevant details for each child in the following format: Column 1 provides the number (by row) of each re-exposed child (left panels) and once-exposed child (right panels). The sex and age of individual children are shown in column 2. Time elapsed from MVC1 to GI endoscopy is provided in column 3. Column 4 identifies the measles-containing vaccine exposure of children and the age in months at which these vaccines were given. The right-hand side of the table provides the identical information as above, for once-exposed children. There was no evidence of celiac disease in any child. Giardia cysts were identified in one once-exposed child (No. 8, Table 1).

Onset of primary (1^o) physical symptoms followed MCV1 in 22 of 23 (96%) once-exposed children and 16 of 22 (73%) re-exposed children. Re-exposed child no. 23 (Table 1) was excluded from this analysis because he received a first MMR at 4 months of age. This was so early and at such variance with all other children that a valid assessment of physical symptoms was deemed impractical. The 1^o physical symptoms included frank diarrhea (4 re-exposed, 7 once-exposed), constipation (2 re-exposed, 5 once-exposed), alternating constipation and diarrhea (4 re-exposed, 3 once-exposed), abdominal pain, often associated with bloating (5 re-exposed, 8 once-exposed), blood per rectum (2 re-exposed, 1 once-exposed), and recurrent mouth ulceration (2 re-exposed). Non-GI symptoms included onset of seizures (1 re-exposed, 1 once-exposed) and gait disturbance with loss of motor skills (6 re-exposed, 7 once-exposed). These neurological features are reported here for interest, given their documented association with GI inflammation [22]. Of the 6 re-exposed children who developed 1^o physical symptoms after MCV2, 5 children suffered onset of 1^o chronic GI problems as described above. One child stopped growing for 18 months; his growth re-commenced only after the introduction of a gluten-free, casein-free (GFCF) diet.

Onset of secondary (2^o) GI symptoms after the second MCV (MCV2) was evident in 11 of 22 (50%) of the re-exposed group; i.e. 11 of the 16 (69%) children who had experienced 1^o GI symptoms following MCV1. Because none of the once-exposed children developed 2^o physical symptoms, it was not possible to calculate a quantitative relative risk estimate. However the difference between re-exposed and once-exposed children for occurrence of 2^o GI symptoms is statistically significant (RR ∞ (infinity) $p<0.0001$). The difference between re-exposed and once-exposed children for occurrence of 2^o fecal and/or urinary incontinence (having previously been continent, i.e., toilet trained) is also statistically significant (RR 2.44; 95% CI, 1.68-3.55).

The 2^o physical symptoms involved chronic exacerbation of pre-existing GI problems and development of novel chronic GI symptoms including: severe constipation (4), diarrhea (3), alternating constipation and diarrhea (2), blood and mucus per rectum (2), recurrent mouth ulceration (3), failure to thrive (2), and fecal incontinence (6). Some children had more than one symptom. Accompanying these GI symptoms were loss of co-ordination with gait disturbance (3), erythema nodosum (1) and joint pains (1). The latter was diagnosed as anti-nuclear antibody-positive juvenile arthritis. One re-exposed child became clumsy with non-epileptiform falling episodes after his initial MMR at 15 months; following his booster MMR at 96 months he was unable to stand at all for 2 weeks, a situation that gradually resolved over several months.

8. Ileo-colonoscopy

The ileum was evaluated in 23 re-exposed and 22 once-exposed children. Ileal LNH was present in 22 of 23 (96%) re-exposed and 21 of 22 (95%) once-exposed children (NS). Severe (grade III) ileal LNH was present in 14 of 23 (61%) re-exposed children and 3 of 22 (14%) once-exposed children (RR 4.46; 1.48-13.43). Of the remaining children, both groups exhibited similar proportions of grades 0-2. The entire colon was visualized in all children. Colonic LNH was seen in 12 of 23 (52%) re-exposed children and 10 of 23 (43%) once-exposed children (NS). There was no predilection for LNH in any particular part of the colon. Features indicative of colonic mucosal inflammation, including erythema, loss of vascular pattern, and mucosal granularity were seen in 12 (52%) re-exposed children, and 14 (61%) once-exposed children (NS). Aphthoid ulceration was seen in two (9%) re-exposed and one (5%) once-exposed child (NS).

9. Histopathology

Details of surgical pathology are provided in Table 2. Pathological changes were patchy and distributed throughout the colon as described previously [4]. Acute inflammation of the colonic mucosa was present in 14 of 23 (61%) re-exposed children and 3 of 23 (13%) once-exposed children (RR 4.67; 1.55 - 14.09) (Table 2). For large bowel biopsies, the proportion with acute inflammation is significantly greater in re-exposed chil-

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dren than in once-exposed children (RR 4.93; 2.15-11.35) (Table 2).

In order to make a valid comparison of the severity of acute inflammation between groups, grades of inflammation were expressed as a proportion of biopsies showing acute inflammation in each group, i.e., where acute inflammation was present, how did its severity compare between groups? Moderate and severe grades were combined for the purposes of statistical analysis since there were no once-exposed children with severe inflammation. The severity of acute inflammation although, greater in re-exposed children than in once-exposed children (RR 4.35; 0.72-26.37) (Table 2), was limited in power by the fact that so few biopsies from once-exposed children had even mild acute inflammation.

There is no significant difference between re-exposed children and once-exposed children for the presence of chronic inflammation

The number of children showing epithelial damage, including disruption of the epithelial basement membrane, crypt architectural changes, and goblet cell depletion, is not significantly different between re-exposed and once-exposed children (RR 1.36; 0.81-2.30) (Table 2). However, the number of biopsies showing epithelial damage is significantly greater in re-exposed children than in once-exposed (RR 1.49; 1.09-2.03) (Table 2).

10. Time-elapsed from MCV1 to endoscopy

Re-exposed and once-exposed groups were matched as closely as possible for time elapsed from MCV1 to endoscopy in order to take into account any progression of disease over time. There is no significant difference between re-exposed and once-exposed children for time elapsed (mean +/- standard deviation: 89 ± 28 months in re-exposed children versus 78 ± 27 months in once-exposed children). However, a mean difference of approximately 11 months may be biologically significant in terms of the disease's natural history.

In order to determine whether time elapsed was independently associated with mucosal pathology, re-exposed and once-exposed groups were merged and then segregated into those (i) with and without acute inflammation, and (ii) those with mild or severe ileal LNH. There is no significant difference in time elapsed between those groups with and without acute inflammation or between those with mild and severe ileal LNH.

11. Discussion

This study tested an a priori hypothesis and the results provide evidence of a re-challenge effect associated with polyvalent measles-containing vaccines, specifically MMR and MR vaccines. The data build upon the growing clinical and basic scientific evidence of an association between autistic-like developmental regression, mucosal immunopathology and MV exposure [3,10,15-17].

Clearly, it would be of interest to examine ASD children who do not have GI symptoms, and ASD children who had received only monovalent measles-containing vaccine in a similar manner. However, in the former case, the ethical constraint against performing invasive procedures on asymptomatic children means that this comparison is not feasible. In the latter case, the fact that all countries with comparable demographics and diagnostic facilities have used MCV over the same period makes this comparison impossible at this time.

A potential shortcoming of this study is that not all expert developmental diagnoses were re-evaluated in our unit. Re-evaluation has been performed in previous studies [3,4,8], and we have no reason, based upon these prior observations, to doubt the accuracy of the original diagnoses. All children studied remain under review by local developmental pediatricians, and we are unaware of any case in which the diagnosis has been revised. Vaccination histories were verified in the study children. It is possible, however, that without access to complete records on all 179 children, because of available resources, some re-exposed children may not have been identified for inclusion in the study.

All children in this study were identified from the same source population, that is, children with a pervasive developmental disorder referred for investigation of GI symptoms. Inclusion of a once-exposed group was considered essential in order to minimize selection and recall biases. One potential problem with this kind of study is that because the conditions of interest (neurodevelopmental and physical symptoms) are often insidious in onset and diagnosis considerably delayed [23], there is rarely a specific acute event to be documented. Physicians may also be reluctant to ascribe a novel event to an adverse vaccine reaction. In addition, documentation was generally of a relatively poor standard. In light of previous experience [24], it was considered that these factors would tend to bias towards the null hypothesis, i.e., no association.

The natural history of the mucosal lesion is not known. We are not yet in a position to say whether the excess of acute inflammation in the re-exposed group is permanent, or whether, as with classical inflammatory bowel disease, it reflects episodic reactivation. We can only say that acute inflammation was more common and had a tendency to greater severity in the re-exposed group. The proportion of biopsies with acute inflammation was also greater in re-exposed children, although this particular statistical analysis assumed that all observations derived from independent cases. This will function to underestimate the observed standard error and leads to the possibility of a Type I error in which the null hypothesis is incorrectly rejected. It is possible that inflammation either progresses or remits over time. However, time-elapsed was not an independent risk factor for pathology, including acute inflammation and LNH, when those children with and without these features were compared.

In order to examine further the relationship of these outcomes, acute inflammation and presence and severity of LNH were independently linked to age at endoscopy in a study of the first 148 ASD children in the source population [25]. There is no significant association between age and any of these mucosal features. It might be anticipated that symptoms and disease severity would be greater in the once-exposed group; these children were not re-vaccinated since, for the majority, parents had made an association between symptoms and MCV1 exposure and therefore, did not have their children re-vaccinated with MCV. This possibility is supported by the observation that 1° physical symptoms were reported in all once-exposed chil-

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dren following MCV1, whereas they were reported only after MCV2 in 6 re-exposed children. This suggests that, if anything, the initial disease ran a milder course in re-exposed children and that, if there were no causal association with MCV, the mucosal pathology should be less severe. This was not the case.

Several aspects of this study strengthen the possibility of a causal association between MCV and the syndrome of autistic-like developmental regression and enterocolitis. First is a challenge re-challenge effect on physical symptoms. Second, is a "biological gradient" [24] seen particularly in the severity of mucosal inflammation. Severe ileal LNH and acute "active" inflammation were more common in re-exposed children. In addition, the risk of an event such as onset of physical symptoms increased with higher frequency of exposure, with primary symptoms starting only after MCV2 in some children. The idiosyncratic nature of the mucosal lesion that, as detailed pathological studies have indicated, is distinct from classical inflammatory bowel disease (Crohn's disease or ulcerative colitis) [8,9,11,12,26], supports a specific etiological process.

The importance placed upon re-challenge in determining causation is evidenced by reports from the IOM's Vaccine Safety Committee, where judgment favoring acceptance of a causal relationship has been based solely on the evidence of one or more convincing case reports. For example, Coulter and Fisher [27] reported one case of hemolytic anemia in a 2-year-old boy, which occurred six days following a fourth dose of DPT vaccine. The boy returned to health until 6 days after his fifth DPT vaccine, when he was re-hospitalized with the identical symptoms that accompanied his initial reaction plus loss of consciousness.

Autistic regression with loss of fecal and urinary continence is consistent with descriptions of childhood disintegrative disorder (CDD) [28]. Although the majority of children had never achieved full continence by the time of their 1º regression, in those re-exposed children who did achieve continence after MCV1, loss of continence and fecal soiling frequently followed MCV2. There are a number of possible reasons for this; a relatively common finding in these children is acquired megarectum, with fecal impaction and overflow or "spurious" diarrhea. This may lead to loss of normal rectal sensation, and fecal incontinence that might operate either independently of, or in concert with, developmental regression. Disturbances in micturition may also follow impaction of the rectum.

Reports of clumsiness, gait disturbance, and ataxia were present in a proportion of these children. Symptoms appeared to be prominent during the earlier phases of regression. This may be relevant to reports of loss of coordination in CDD [28], and gait disturbance and ataxia following MMR as reported by Plesner *et al.* [29,30]. In Denmark this association had not been detected with any other vaccine administered to children of the same age prior to the introduction of MMR in 1987, indicating that a novel adverse event might be associated with the combined MMR vaccine, rather than the monovalent component vaccines. In a recent follow up of Denmark's mandatory passive reporting system, Plesner confirmed this association and indicated that more severe ataxias following MMR may be associated with residual cognitive deficits in some children [30]. It would be of value to establish the nature of these residual deficits.

Epidemiological studies that have examined the possible MMR-autism association have concluded that the data provide no evidence in support of this hypothesis [31-35]. These studies have been challenged on a number of counts including inappropriate methodology [36], lack of statistical power and lack of a control group [37,38], indiscriminate diagnostic groupings, [39] and non-disclosure of relevant data [40]. Reanalysis of the data of Dales *et al.* [34] and Madsen *et al.* [41] has, in fact, identified a positive association for some children [42,43].

Clearly, meaningful epidemiological studies should test a priori hypotheses that derive from all clues evident in the clinical histories of affected children. Thus far, this has not happened.

A crucial question relates to what makes a child susceptible to a possible adverse event to a MCV. Potential risk factors are beginning to emerge in the histories of affected children including: familial autoimmunity, pre-existing dietary allergy/intolerance, vaccination with MCV while unwell (including current or recent antibiotic administration), and receipt of multiple simultaneous vaccine antigens, with the associated potential for immunological interference [43-45], particularly for mumps upon measles virus [44].

The growing burden of infant vaccines may increasingly skew the immune response away from optimal antiviral immunity towards a dominant T-helper cell type 2 repertoire [46]. The rapid increase in numbers of children with dietary allergy—itself associated with reduced CD8 cell numbers, prolonged viral infections, and familial autoimmunity [47] with increasing infant exposure to heavy metal toxicity and antibiotic use over the last 10-15 years—suggests that the number of children who may be at risk of aberrant responses to atypical infectious challenges will have risen in the last decades. A likely autoimmune component to the pathogenesis of regressive autism [9,25] suggests that any causal association with MCV would lead to a continuing upward trend in incidence after vaccine introduction, in developed-world but not developing-world populations, in parallel with other autoimmune lesions.

In summary, this study confirms that there is a challenge re-challenge effect of MMR/MR upon GI symptoms and ileocolonic pathology in these children with pervasive developmental disorder, principally autism. In accordance with previous findings of the IOM, this constitutes evidence suggestive of causality.

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Table 1. A Summary of the demographic details and Measles-containing vaccine exposures in re-exposed and once-exposed children*

	Re-exposed			Once-exposed		
No.	Sex + age (months) at colonoscopy	Interval (months) vaccination to colonoscopy	Vaccine + age (months) at vaccination	Sex + age (months) at colonoscopy	Interval (months) vaccination to colonoscopy	Vaccine + age (months) at vaccination
1	M 192	180	MVM 12 MR 168	M 180	164	MVM 16
2	M 116	101	MMR 15 MMR 49	M 113	101	MMR 12
3	F 113	95	MMR 18 MR 70	F 58	45	MMR 13
4	M 91	78	MMR 13 MMR 49	M 96	82	MMR 14
5	M 134	119	MMR 15 MR 72	M 116	102	MMR 14
6	M 84	72	MMR 12 MR 53	M 80	66	MMR 14
7	M 91	77	MMR 14 MMR 74	M 8	82	MMR 14
8	F 136	122	MMR 14 MR 35	F 8	83	MMR 13
9	M 84	66	MMR 18 MR 53	M 79	66	MMR 13
10	M 120	102	MMR 18 MMR 88	M 112	97	MMR 15
11	M 84	68	MMR 16 MMR 30	M 77	61	MMR 16
12	M 75	62	MMR 13 MMR 71	M 61	48	MMR 13
13	M 81	69	MMR 12 MMR 48	M 72	57	MMR 15
14	M 134	121	MMR 13 MR 73	M 112	99	MMR 13
15	M 84	72	MMR 12 MMR 42	M 79	64	MMR 15
16	M 173	122	MMR 51 MR 108	M 112	97	MMR 15
17	M 87	73	MMR 14 MR 46	M 99	74	MMR 25
18	M 84	70	MMR 14 MMR 52	M 65	52	MMR 13
19	M 72	60	MMR 12 MMR 48	M 60	44	MMR 16
20	M 108	94	MMR 14 MR 59	M 111	92	MMR 19
21	M 84	71	MMR 13 MMR 76	M 84	72	MMR 12
22	M 84	70	MMR 14 MR 58	M 61	47	MMR 14
23	M 98	94	MMR 4 MMR 15	M 116	102	MMR 14

* MMR is measles-mumps-rubella vaccine; MR is measles-rubella vaccine, MVM is monovalent measles-containing vaccine

Table 2. Details of surgical pathology

Pathological feature	Patient group	No. of patients & (Total No. Biopsies)	Cases showing feature No. (%)	Biopsies showing feature No. (%)	Severity of inflammation expressed as No. & (%) of biopsies showing feature		
					Severe	Moderate	Mild
Acute inflammation	*Re-Exposed*	23 (96)	14 (61)	29 (30)	2 (7)	19 (66)	8 (28)
	Once-Exposed	23 (98)	3 (13)	6 (6)	0 (0)	1 (17)	5 (83)
Chronic inflammation	*Re-Exposed*	23 (96)	20 (87)	59 (60)	2 (3)	15 (25)	42 (71)
	Once-Exposed	23 (98)	19 (83)	58 (59)	2 (3)	13 (22)	39 (67)
Eosinophil infiltration	*Re-Exposed*	23 (96)	15 (65)	24 (25)	4 (17)	5 (21)	15 (63)
	Once-Exposed	23 (98)	8 (35)	18 (19)	4 (22)	5 (28)	7 (39)
Epithelial changes	*Re-Exposed*	23 (96)	15 (65)	54 (56)	0 (0)	1 (2)	52 (96)
	Once-Exposed	23 (98)	11 (48)	37 (38)	0 (0)	0 (0)	36 (97)

Note: The severity (mild, moderate, severe) of each feature is presented as the number of biopsies in each category and the (%) of the total number of biopsies showing this feature. Percentage values are rounded down when <0.5% and up when ≥0.5%) For the purpose of statistical comparison, moderate and severe grades were treated as one category since no once-exposed children had severe acute inflammation.

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