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Classification of measles breakthrough cases in an elimination setting using a comprehensive algorithm of laboratory results: why sensitive and specific IgM assays are important

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Abstract

Objective: In 2006, a measles outbreak occurred in Catalonia (Spain), six years after endemic measles was declared eliminated. This study aimed to classify 19 confirmed measles breakthrough cases (BC) using a high-performance avidity assay developed in 2010.

Methods: Serum specimens were tested by indirect IgG, indirect IgM, capture IgM enzyme immunoassay, an endpoint-titer IgG avidity assay, and a plaque reduction neutralization assay. Serology and RNA detection results were combined in an algorithm for measles confirmation and classification of breakthrough cases and analyzed with clinical and epidemiological data.

Results: Of 19 samples, thirteen (68%) were conclusive with the classification of BCs, and six (32%) had false-positive IgM results on an indirect-format assay; they were classified as rash and fever illness of undetermined etiology. BCs were primary vaccine failures (seven or 54%), secondary vaccine failures (four or 31%), and two (15%) could not be classified.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval statement

The CDC Human Research Protections Office determined that this study was not human subjects research and not subject to CDC Institutional Review Board review.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

Conclusions: In measles elimination settings, high-performing assays and a comprehensive algorithm of laboratory results (IgG, IgM, and RNA detection), including IgG avidity and PRN results when necessary, can assist in accurate laboratory confirmation and classification of suspected measles cases for surveillance. Highly specific IgM assays are required to minimize the number of false-positive results.

Keywords

Measles; breakthrough case; avidity assay; vaccine failure; elimination; case classification

Introduction

Sporadic measles outbreaks may still occur in elimination settings due to international travel from measles-endemic areas, and they may occasionally involve individuals with a documented vaccination history (breakthrough cases, or BCs) (Patel et al., 2019). BCs are due to an absence of a measles-specific immune response to the vaccine (primary vaccine failure, PVF) or to a measles-specific immune response that is insufficient to protect against measles (secondary vaccine failure, SVF) (Paunio et al., 2003). This classification, PVF vs. SVF, is essential to characterize BCs and help understand their role in measles outbreaks in elimination settings (Moss, 2018).

Materials and Methods

We retrospectively classified measles BCs from an outbreak in Catalonia (Spain) that occurred from August 2006 to July 2007, six years after endemic measles was declared eliminated in that region (Torner et al., 2007, WHO, 2017). Measles incidence in Catalonia, vaccination coverage, and measles, mumps, and rubella (MMR)-1 and MMR-2 schedules are described elsewhere (Dominguez et al., 2008). There were 381 confirmed outbreak cases, 38 of which were identified as BCs because they fulfilled the measles clinical case definition (maculopapular rash, fever > 38 °C, and at least one of cough, conjunctivitis, or coryza), and receipt of 1 or 2 doses of MMR (documented on patients' vaccination certificates or on health records at the primary care registers). These BCs were laboratory confirmed by a measles-specific IgM indirect-format enzyme immunoassay (EIA) (Vircell, Granada, Spain; 98% specificity, 100% sensitivity per kit's insert) using serum and/or by RT-PCR using urine (Dominguez et al., 2008, Torner et al., 2007). Nineteen serum samples from 19 patients with sufficient volume for additional testing were shipped frozen to the US CDC and stored at −20 °C until tested. Epidemiological, clinical, and laboratory data obtained for the 19 BCs are described (Tables 1 and 2). At CDC, specimens were tested for measles IgG antibodies by EIA (Trinity Biotech, Jamestown, NY), and negative specimens were tested on a more sensitive IgG EIA (Zeus Scientific, Branchburg, NJ) (Latner et al., 2020). Additional measles testing included IgG avidity if IgG positive, IgM by a capture-format EIA (> 99% specificity, 100% sensitivity per (Hummel et al., 1992)), and neutralizing antibodies by a plaque reduction neutralization (PRN) assay; all assays were performed as previously described (Cohen et al., 2007, Hummel et al., 1992, Mercader et al., 2012, Sowers et al., 2016). We used the patient's clinical and epidemiological data (Table 1) and the results obtained at CDC for case confirmation and classified BCs as PVF, SVF, or

unclassified vaccine failure (UVF) following the algorithm described in Table 2. BCs were re-classified as rash and fever illness (RFI) of unknown etiology in vaccinated individuals if laboratory results were inconclusive for case confirmation. For differential diagnosis, we tested specimens for rubella IgM (Diamedix Corporation, Hialeah, FL) and parvovirus B19 IgM (Diasorin, Stillwater, MN) by EIAs; all commercial EIAs were performed and analyzed following product inserts.

Results

We analyzed 19 samples; thirteen (68%) were conclusive with the classification of BCs (Table 2). The remaining six (32%) samples had inconclusive results with false-positive IgM results on the indirect-format assay and were re-classified as RFIs; all six samples were rubella and parvovirus B19 IgM negative. The six samples that had an IgM positive result by the indirect-format assay were not retested on the indirect-format assay. They were retested on the capture-format assay, and the results we obtained by this test were IgM negative. We analyzed all laboratory, epidemiological and clinical data for these samples and concluded that the samples had a false-positive IgM result by the indirect-format assay. Among the thirteen confirmed BCs, there were seven (54%) PVFs, 4 (31%) SVFs, and two (15%) UVF. Patients 1–5 (PVFs) had measles IgG negative results; outbreak response immunization doses were administered to patients 1 and 2 < 11 months of age, and to patient 5, five days before rash onset. Patient 11 (SVF) was an otherwise healthy 19-month-old child with MMR-1 at age 15 months.

Discussion

Our results are a reminder of the importance of accurate laboratory confirmation and classification of suspected measles cases for surveillance. Well-validated, highly sensitive, and specific IgM assays are crucial in elimination settings, where the rate of false-positive IgM results is high, as demonstrated here with 32% of false-positive IgM results (Moss, 2018). Testing for measles-specific IgG, IgM, and RNA is essential to increase confirmation accuracy.

BCs occur, and their classification is critical to understand the cause of outbreaks in highly vaccinated populations (Moss, 2018). More than half of the thirteen identified BCs were PVFs, and 31% were SVFs illustrating the utility of IgG avidity testing in case classification (Paunio et al., 2003). Specifically in this outbreak, possible factors in PVFs included failure to mount a humoral immune response to measles vaccine; and in SVFs, a) failure to maintain protection against measles > 8 years after MMR (patients 8–10), and b) failure to attain an initial robust immunologic response to the measles vaccine, resulting in loss of protection to measles four months after MMR-1 (patient 11).

SVF cases may have modified presentations that are not easily recognized (Mercader et al., 2012, Moss, 2018, Sowers et al., 2016). It is essential to identify such cases because SVFs can transmit measles, although at reduced efficiency (Mercader et al., 2012, Moss, 2018, Sowers et al., 2016). A better understanding of eliminating measles outbreaks may be achieved with high-performing assays and a comprehensive algorithm of laboratory

results (IgG, IgM, and RNA), including IgG avidity and PRN testing for confirmation and classification of BCs, when necessary. Highly specific IgM assays are required in elimination settings.

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Table 1

Clinical and epidemiological characteristics of measles cases confirmed during a measles outbreak in Barcelona (Spain), 2006.

Patient	Age in years (mo.)	MMR doses[a]	Age at MMR-1	Age at MMR-2	Symptoms[b]	Epidemiologically linked
1	0.8 (9)	1[c]	7 mo.		Cough	Yes
2	0.9 (11)	1[c,d]	Unknown		Cough, otitis, diarrhea	Yes
3	2	1[d]	17 mo.		Cough	Yes
4	4	1[d]	3 yr.		Cough	No
5	1.2 (14)	1[c,d]	14 mo.		Cough, coryza	Yes
6	2	1[d]	15 mo.		Cough	No
7	1.4 (17)	1[d]	15 mo.		Coryza	No
8	26	1	2 yr.		Coryza	Yes
9	13	1	4 yr.		Coryza	Yes
10	11	2	15 mo.	4 yr.	Cough	No
11	1.6 (19)	1	15 mo.		Cough, otitis	Yes
12	26	1[d]	11 yr		Cough	No
13	3	1	15 mo.		Cough	No
14	2	1	17 mo.		Coryza	No
15	3	1	15 mo.		Cough	No
16	12	2	15 mo.	5 yr.	Cough	No
17	3	1	16 mo.		Cough, coryza	No
18	2	1	18 mo.		Cough	No
19	2	1	16 mo.		Coryza	No

MMR: measles, mumps, rubella vaccine; mo.: months yr.: years;

[a] MMR was scheduled at age 15 months (since 1981), and MMR-2 at eleven years (from 1988 to 1999) and at four years (since 1999) [5]

[b] All patients had rash and fever.

[c] Patient received an outbreak response immunization (ORI) at 35 (patient 1), five (patient 5), and unknown (patient 2) days before rash onset.

[d] Patient was negative for rubella IgG; all other patients were IgG positive for rubella. All patients were rubella IgM negative.

Table 2

Retrospective case classification of measles breakthrough cases identified initially during a measles outbreak in Barcelona (Spain), 2006

Patient	Serum collection (days)	IgM result Indirect	Capture	IgG ISR[a]	Avidity result	PRN titer	Urine collection (days)	RT-PCR result	Case classification[b]	Comments
1	7	P	P	1.0	NT	1,936	NCD	NT	PVF	Possible interference of maternal antibodies and immaturity of the immune system at vaccination [20, 21].
2	8	N	P	0.5	NT	1,361	8	p[c]	PVF	
3	NCD	NT	P	0.5	NT	1,022	0	p[c]	PVF	
4	0	P	P	0.4	NT	630	0	P	PVF	
5	2	P	N	0.4	NT	49	2	p[c]	PVF	Possibly due to insufficient time for seroconversion after receiving an ORI (five days prior to rash onset).
6	9	P	P	2.4	L	3,568	2	NT	PVF	
7	8	P	P	3.1	L	2,230	1	p[c]	PVF	
8	0	P	P	5.3	H	113,111	NCD	NT	SVF	
9	11	P	P	5.5	H	146,175	1	p[c]	SVF	
10	6	P	P	5.9	H	109,934	0	P	SVF	
11	2	P	P	1.9	H	2,327	2	P	SVF	One possible explanation is that a full vaccine dose was not injected.
12	2	P	P	2.3	I	6,398	2	p[c]	UVF	
13	8	P	p[d]	5.2	H	4,481	8	N	UVF	
14	2	P	N	3.0	I	1,048	3	N	RFI	
15	8	P	N	3.3	H	486	NCD	NT	RFI	
16	7	P	N	1.9	H	135	NCD	NT	RFI	Though protected, patient 16 had PRN titers just over the cut-off (>120 mIU/mL) eleven years after MMR-1 and seven years after MMR-2.
17	9	P	N	4.3	H	312	NCD	NT	RFI	
18	3	P	N	3.0	H	1,607	NCD	NT	RFI	
19	4	P	N	4.7	H	1,801	NCD	NT	RFI	

ISR: immune status ratio; PRN: plaque reduction neutralization; NT: not tested; ORI: Outbreak Response Immunization; NCD: no collection date; N: negative result; L: low avidity; H: high avidity; I: intermediate avidity.

[a]Measles IgG positive result if ISR ≥ 1.1

[b]Case classification: Primary vaccine failure if IgM and/or RT-PCR were positive, and IgG was negative or low-avidity; Secondary vaccine failure if results were IgM positive, RT-PCR positive, and/or had PRN titers ≥ 40,000 mIU/mL and IgG was high-avidity; Unclassified vaccine failure if a) IgM and/or RT-PCR were positive, IgG was intermediate-avidity and PRN titers < 40,000 mIU/mL, or b) IgM results were borderline low positive, and RT-PCR, avidity and PRN results were inconclusive for classification.

[c]Measles virus genotype D4

[d]Borderline positive by the measles capture-format IgM test; false-positive IgM result suspected.