Distinguishing between primary measles infection and vaccine failure reinfection by IgG avidity assay

R. Hamkar,[1] M. Mahmoodi,[1] R. Nategh,[1] K.N. Jelyani,[1] M.B. Eslami[1] and T. Mohktari-Azad[1]

التمييـز بين العدوى الأولية بالحصبة وبين عودة العدوى بسبب فشل اللقاح، باستخدام المقايسة المناعية ضد الغلوبولين المناعي G

رسول همكار، محمود محمودي، رخشندة ناطق، كرامت نوري جيلاني، محمد باقر إسلامي، طلعت مختاري آزاد.

الخلاصــة: تم في هذه الدراسة، التي أُجريت في جمهورية إيران الإسلامية، تقيـــيم 365 حالـة حـصبة، للتفريـق بـين العدوى الأولية بالحصبة وبين عودة العدوى بسبب الفشل الثانوي للقاح. وأعيدَ فحص جميـع الحـالات الـتي سـبق تأكيد إصابتها بتحرِّي الغلوبولين المناعي M بواسطة تحرِّي الغلوبولين المناعي G. ولوحظت استجابة مناعيـة ثانويـة لدى 18.4٪ من المرضى. وأبدى جميع المرضى غير المطعَّمين (16.7٪) استجابة مناعية أولية. ومن بين 244 مريضاً ثبت تطعيمهم من واقع السجلات، أبدى 75.8٪ منهم استجابة مناعية أولية، وأبدى 24.2٪ منهم استجابة مناعية ثانوية، مما يشير إلى فشل ثانوي للقاح. ولوحظ أن جميع حالات عودة العدوى بالحصبة (99٪) قـد شـوهدت في المرضى الذين هم أكبر من سن 10 سنوات، مما يُوصى معه بتطعيم الأطفال الذين هم في سن العاشرة.

ABSTRACT In this study in the Islamic Republic of Iran 365 measles cases were evaluated to distinguish between primary infection with measles and reinfection due to secondary vaccine failure. All cases previously confirmed by detection of specific IgM were tested for IgG avidity. A secondary immune response was seen in 18.4% of patients. All unvaccinated patients (16.7%) showed a primary immune response. Of 244 patients with documented vaccination, 75.8% showed a primary immune response and 24.2% showed a secondary immune response, thereby indicating a secondary vaccine failure. Almost all measles reinfections (99%) were seen in patients >10 years old, indicating that vaccination for 10-year-old children is recommended.

Distinguer la primo-infection rougeoleuse de la réinfection après un échec vaccinal grâce au test d'avidité des IgG

RÉSUMÉ Dans cette étude en République islamique d'Iran, 365 cas de rougeole ont été évalués pour distinguer une primo-infection rougeoleuse de la réinfection due à l'échec de la vaccination secondaire. Le test d'avidité des IgG a été effectué pour tous les cas confirmés auparavant par la détection des IgM spécifiques. Une réponse immunitaire secondaire a été observée chez 18,4 % des patients. Tous les patients non vaccinés (16,7 %) ont montré une réponse immunitaire primaire. Sur les 244 patients pour lesquels la vaccination était documentée, 75,8 % montraient une réponse immunitaire primaire et 24,2 % une réponse immunitaire secondaire, indiquant ainsi un échec de la vaccination secondaire. Presque toutes les réinfections (99 %) étaient observées chez des patients âgés de plus de 10 ans, ce qui indique que la vaccination des enfants de 10 ans est recommandée.

[1]School of Public Health, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran (Correspondence to R. Hamkar: rhamkar@sina.tums.ac.ir).

Introduction

Exposure to the measles virus is steadily decreasing worldwide due to mass vaccination, and millions of people are protected solely by immunity induced by attenuated vaccines [1,2]. However, there are doubts about the quality and duration of vaccine-induced immunity [3,4]. Two kinds of failures, primary failure and secondary failure, are attributed to measles vaccine. Primary failure indicates that the vaccine has not taken and does not induce any immunological response, while secondary failure indicates that vaccine-induced immunity against measles has waned in the years after vaccination [5–7]. Low vaccination age is known to adversely affect measles vaccine efficacy, mainly due to the presence of maternal antibodies, and this is regarded as primary vaccine failure [3]. Secondary vaccine failures, however, are largely attributed to the waning of primary antibody response over time [3,5–8].

As the serological status preceding measles is usually unknown, it is difficult by conventional means to establish the occurrence of secondary vaccine failures (waning and/or incomplete immunity) and related factors [3]. In a study of vaccinated students who donated blood just before infection, low antibody levels increased the risk of measles [9]. There is a report of 4 health care workers who contracted measles despite prior successful vaccination [10]. Other case reports of secondary vaccine failure have been published, including one of a Chinese patient who seroconverted after vaccination at 8 months [3]. In a 10-year follow-up study of children in Canada inoculated at 12 months of age, 6%–9% developed clinical measles [11]. In a study in 1996–97 in the Islamic Republic of Iran 9% of measles cases were among previously vaccinated patients [7].

The avidity (functional affinity) of immunoglobulin (Ig)G antibodies has long been known to distinguish primary from secondary immune responses against many antigens. Virus-specific high-avidity antibodies are generally associated with pre-existing B-cell memory, whereas low-avidity IgG is an indication of the primary immune response [6,12–15]. Thus, avidity measurement can be used to assess the success of measles vaccination [16] and offers a way of assessing the type of vaccine failure without knowledge of prior antibody status [15,17,18].

In this study in the Islamic Republic of Iran, conducted in 2003, we used IgG avidity assay to analyse sera from laboratory-confirmed measles patients, in order to determine how many were cases of reinfection (i.e. high-avidity antibodies) and how many were cases of primary infection (i.e. low-avidity antibodies).

Methods

Samples

Before the measles/rubella mass vaccination programme in December 2003, more than 5000 serum samples of suspected measles cases were collected annually in all parts of the Islamic Republic of Iran, of which about 55% were laboratory-confirmed as measles infection. In this study the low avidity anti-measles IgG panel and study group sera were selected from laboratory-confirmed Iranian measles cases during March 2002 to March 2003, while the high avidity anti-measles IgG panel sera were collected from normal people aged 11 to 15 years old in Tehran, who had been vaccinated twice for measles at 9 and 15 months of age during routine vaccination programmes in the Islamic Republic of Iran.

Low-avidity anti-measles IgG panel

Sera were collected from laboratory-confirmed measles patients aged 9 months to 2 years old. All sera were tested for anti-measles IgM and IgG antibodies (Enzygnost Anti-Measles Virus IgM and IgG, Dade Behring, Marburg, Germany). A total of 45 serum samples which were anti-measles IgM- and IgG-positive were selected. Based on the hypothesis that measles patients who were less than 2 years of age had been exposed to the measles virus and experienced a primary infection, and were not re-infected by measles virus, these sera were regarded as the low-avidity anti-measles IgG panel.

High-avidity anti-measles IgG panel

Sera were collected from normal people aged 11 to 15 years old, who had been vaccinated twice for measles at 9 and 15 months of age during routine vaccination programmes in the Islamic Republic of Iran. All sera were tested for anti-measles IgG and IgM antibodies (Enzygnost Anti-Measles Virus IgG and IgM, Dade Behring, Marburg, Germany). A total of 45 serum samples which were anti-measles IgM negative and anti-measles IgG positive were selected. Based on the hypothesis that at least 10 years after measles vaccination they would have high-avidity IgG against measles virus, these sera were regarded as the high-avidity anti-measles IgG panel.

Study group sera

Between March 2002 and March 2003, sera from acute measles cases were collected as part of the measles surveillance programme in the Islamic Republic of Iran. At the time of serum sample collection, a questionnaire about personal data and vaccination status was completed. Cases were confirmed as measles when testing for anti-measles IgM using enzyme immunoassay (EIA) kits (Dade Behring, Marburg, Germany)

gave positive IgM results. Then all anti-measles IgM positive sera were tested for anti-measles IgG, and negative cases were excluded from the study. In total 365 anti-measles IgM and IgG positive cases from various age groups were selected.

Avidity measurement

All sera including low- and high-avidity anti-measles IgG panels and study groups were subjected to anti-measles IgG avidity assay. The avidity of IgG for measles virus was measured by a protein-denaturing EIA where the antibodies were first allowed to bind to the virus antigen, followed by elution with or without 6 M urea [6,9,19,20].

In preliminary experiments we compared 2 dilutions of sera for the avidity assay. Each sample was tested at 2 dilutions (1:21, 1:42), and each dilution at 4 replicates. For each replicate a single serum dilution (1:21 or 1:42) of the kit's serum diluents was applied to each of 4 wells on 1 row of EIA plates (2 wells of measles antigen positive-coated and 2 wells of measles antigen negative-coated). After incubation for 1 h, test plates were washed 4 times according to the EIA kit procedures. Then 2 wells (1 antigen-positive and 1 antigen-negative) were soaked for 5 min in wash buffer and 2 others for 5 min in wash buffer containing 6 mol/L urea. Fresh buffers were applied and the soaking step was carried out twice more. The plates were then washed 4 times with wash buffer. Then the test was continued according to the kit. The remaining specific antibody was then detected according to the EIA kit procedure. An avidity index (AI) was calculated from the optical density (OD) in the wells: AI = (ΔOD with urea/ΔOD with wash buffer) × 100

In almost all cases the 2 dilutions gave similar results and higher reproducibility rate (99%) at 1:21 dilution; therefore all sera were assayed at this dilution.

Controls

Three controls were employed for testing EIA plates: serum samples containing strong high-avidity, weak high-avidity and low-avidity anti-measles IgG antibody. The kit's positive and negative controls were also applied.

Statistical methods

All AI values obtained from testing of both high and low-avidity panels were analysed by the classification and regression tree (CART) statistic method [21] using SPLUS software. AI values < 58.65% were classified as low-avidity and AI ≥ values 58.65% were classified as high-avidity. The misclassification error rate was zero (0/90).

Other statistical analyses were undertaken using *Epi-info*, version 3.2.2. The laboratory findings and personal data among study groups were compared using the chi-squared test [22].

Results

The distribution of serum samples from the study group by age and measles vaccination status is given in Table 1. All samples were from laboratory-confirmed measles cases (anti-measles IgM positive) from all age groups. Of the 365 patients, 61 (16.7%) had not been vaccinated, 244 (66.8%) had received 1 or 2 doses of measles vaccine and 60 (16.4%) had unknown vaccination status.

Overall, 67 (18.4%) measles cases confirmed by a positive IgM test exhibited high-avidity IgG representing secondary immune response to measles (i.e. secondary vaccine failure), while 298 (81.6%) of them exhibited a primary immune response indicating primary measles infection (Table 2).

The distribution of anti-measles low- and high-avidity IgG among the study group by age is presented in Table 2. Low-avidity anti-measles IgG was detected in 100% of patients aged < 5 years and this proportion decreased with increasing age to only 72.0% of those aged > 25 years ($P < 0.001$). High-avidity anti-measles IgG was detected in a significantly higher proportion of patient aged > 25 years and 20–25 years respectively (28.0% and 26.7%), while only 1 case (3.2%) was found in the 5–10 years age group and none in < 5 years age group ($P < 0.001$).

Table 1 **Distribution of anti-measles IgM-positive cases by age and measles vaccination status**

Age (years)	Total tested	Vaccination status				No. of doses (known vaccination status)		
		Unknown		Known		0	1	2
	No.	No.	%	No.	%	No.	No.	No.
< 5	56	12	21.4	44	78.6	18	12	14
5–10	31	7	22.6	24	77.4	5	12	7
10–15	55	11	20.0	44	80.0	3	13	28
15–20	93	11	11.8	82	88.2	11	47	24
20–25	105	8	7.6	97	92.4	19	64	14
> 25	25	11	44.0	14	56.0	5	9	0
Total	365	60	16.4	305	83.6	61	157	87

Table 2 **Distribution of low- and high-avidity anti-measles IgG in the study group by age**

Age (years)	Total tested No.	Anti-measles IgG			
		Low-avidity		High-avidity	
		No.	%	No.	%
< 5	56	56	100.0	0	0
5–10	31	30	96.8	1	3.2
10–15	55	48	87.3	7	12.7
15–20	93	69	74.2	24	25.8
20–25	105	77	73.3	28	26.7
> 25	25	18	72.0	7	28.0
Total	365	298	81.6	67	18.4

$\chi^2 = 25.17$; P < 0.001.

Discussion

In 1990 the World Health Organization (WHO) set a goal for eliminating measles in the Eastern Mediterranean Region, including the Islamic Republic of Iran, by the end of 2010 [23]. However, in 2003, before the mass campaign programme for measles vaccination, more than 4000 cases of laboratory-confirmed measles were reported in the Islamic Republic of Iran, most of whom had been vaccinated against measles [24]. Vaccine-induced protection has been well-documented to be less durable and less robust than naturally-acquired immunity against measles virus [25], and high occurrence of symptomatic but mild measles due to secondary vaccine failures has been found among measles patients vaccinated over a decade ago, especially among those who were revaccinated [6].

The distribution of anti-measles low- and high-avidity IgG among the study group by vaccination status is shown in Table 3. None of the 61 unvaccinated patients (0%) showed high-avidity anti-measles IgG and their measles is regarded as a primary infection. Of the 244 patients with documented vaccination, 185 (75.8%) showed a primary immune response and 59 (24.2%) showed a secondary immune response, thereby indicating a secondary vaccine failure.

A significantly higher proportion of those who received 2 doses of measles vaccines during the vaccination programme showed high-avidity anti-measles IgG (34.5%) (P < 0.001). This figure was 18.5% for those who received a single dose of measles vaccine (Table 3).

Understanding the reasons for primary and secondary vaccine failures is important for the evaluation of measles control programmes in developing countries. A high proportion of primary vaccine failures in vaccinated patients with measles can indicate, for instance, problems with improper vaccine handling. However, the introduction of enhanced diagnostic tests for IgM detection such as IgM-capture EIA, with results which may be positive for patients with measles reinfection due to secondary vaccine failure, has highlighted the difficulty in differentiating between pri-

Table 3 **Distribution of low- and high-avidity anti-measles IgG in the study group by vaccination status**

Anti-measles IgG	Total tested No.	Vaccination status				No. of doses (known vaccination status)					
		Unknown		Known		0		1		2	
		No.	%	No.	%	No.	%	No.	%	No.	%
High-avidity	67	8	13.3	59	19.3	0	0	29	18.5	30	34.5
Low-avidity	298	52	86.7	246	80.7	61	100.0	128	71.5	57	65.5
Total	365	60	100.0	305	100.0	61	100.0	157	100.0	87	100.0

$\chi^2 = 27.49$; P < 0.001.

mary infection or reinfection due to primary and secondary vaccine failure [5,6,26–28]. Measles reinfection due to secondary vaccine failure is probably more common than suggested by studies relying on specific IgM [6], because measles-specific IgM is also inducible by reinfection [26].

The estimation of IgG antibody avidity is useful for identifying primary and secondary immune responses, but there have been few reports of its use during measles outbreaks [5]. The results of the present study, which showed that 18.4% of 365 measles cases confirmed by a positive IgM test mounted a secondary immune response, provide further evidence that the presence of IgM cannot be used as a reliable indicator of a primary immune response [5].

As expected, all unvaccinated subjects showed a primary immune response, validating the information given by the IgG avidity test. Analysis of the number of vaccine doses and the type of vaccine failure showed that the measles patients who had received 2 vaccine doses had a rate of primary vaccine failure which is significantly lower than that of the group of patients who had received a single vaccine dose. However the high proportion of cases analysed in the present study that were associated with a secondary immune response, suggests that secondary vaccine failures also played an important role in the measles outbreaks during 2003 in the Islamic Republic of Iran. The rate of measles reinfection due to secondary vaccine failure in the twice-vaccinated group was significantly higher than that of the group of patients who had received a single vaccine dose, and this confirms other studies [5,6]. We found no cases of measles reinfection in the unvaccinated group, indicating that reinfection occurred among vaccinated people due to waning of

protective immunity against measles; this is also compatible with the results of other studies [25,26,28].

Until recently [6], there has been a lack of convincing evidence for waning immunity after measles vaccination without the boosting effect of natural infection [5,6]. If immunity is waning, we would expect to see a higher occurrence of a high-avidity response with increasing time since vaccination. This was the case in our study, where measles reinfection due to secondary vaccine failure significantly increased with increasing age. Almost all cases of measles reinfection (99%) were seen in the > 10 years age group, indicating that vaccine-induced immunity could wane after about 10 years and for achieving good performance in measles virus elimination a further dose of vaccine for 10-year-old children should be recommended.

During December 2003 to January 2004, a mass campaign for measles vaccination was conducted in the Islamic Republic of Iran, and populations aged between 5–25 years old were vaccinated by measles/rubella vaccine. The results of the present study suggest that vaccination of the 25–30-year-old population should be recommended, and that after 5 years all 10-year-old children receive a booster dose of measles vaccine.

Acknowledgements

References

1. De Quadros CA et al. Measles elimination in the Americas. *Journal of the American Medical Association*, 1996, 275:224–9.

2. Rosenthal SR, Clements CJ. Two-dose measles vaccination schedules. *Bulletin of the World Health Organization*, 1993, 71:421–8.

3. Markowitz LE et al. Duration of live measles vaccine-induced immunity. *Pediatric infectious disease journal*, 1990, 9:101–10.

4. US Preventive Services Task Force. Immunizations and chemoprophylaxis. Childhood immunizations. In: DiGuiseppi C, Atkins D, Woolf SH, eds. *Guide to clinical preventive services*, 2nd ed. Baltimore, Williams and Wilkins, 1996:767–90.

5. Pannuti SC et al. Identification of primary and secondary measles vaccine failures by measurement of immunoglobulin G avidity in measles cases during the 1997 Sao Paulo epidemic. *Clinical and diagnostic laboratory immunology*, 2004, 11:119–22.

6. Paunio M et al. Secondary measles vaccine failures identified by measurement of IgG avidity: high occurrence among teenagers vaccinated at a young age. *Epidemiology and infection*, 2000, 124(2):263–71.

7. Mokhtari-Azad T et al. Epidemiologic features of measles in Iran, during 1996–1997. *Hakim research journal*, 1998, 3(1):123–9.

8. Aaby P et al. Vaccinated children get milder measles infection: a community study from Guinea-Bissau. *Journal of infectious diseases*, 1986, 154:858–63.

9. Chen RT et al. Measles antibody: re-evaluation of protective titers. *Journal of infectious diseases*, 1990, 162:1036–42.

10. Ammari LK, Bell LM, Hodinka RL. Secondary measles vaccine failure in health-care workers exposed to infected patients. *Infection control and hospital epidemiology*, 1993, 14:81–6.

11. Mathias RG et al. The role of secondary vaccine failure in measles outbreaks. *American journal of public health*, 1989, 79:475–8.

12. Binley MB et al. An investigation of the high-avidity antibody response to glycoprotein 120 of human immunodeficiency virus type 1. *AIDS research and human retroviruses*, 1997, 13:1007–15.

13. Soderlund M et al. Accurate serodiagnosis of B19 parvovirus infections by measurement of IgG avidity. *Journal of infectious diseases*, 1995, 171:710–3.

14. Hedman K, Seppala I. Recent rubella virus infection indicated by a low avidity of specific IgG. *Journal of clinical immunology*, 1988, 8:214–21.

15. Tuokko H. Detection of acute measles infections by indirect and mu-capture enzyme immunoassays for immunoglobulin M antibodies and measles immunoglobulin G antibody avidity enzyme immunoassay. *Journal of medical virology*, 1995, 45:306–11.

16. De Souza VA et al. Enzyme-linked immunosorbent assay-IgG antibody avidity test for single sample serologic evaluation of measles vaccines. *Journal of medical virology*, 1997, 52:275–9.

17. Matsuzono Y et al. Measles encephalomyelitis in a patient with a history of vaccination. *Acta paediatrica japonica*, 1995, 37:374–6.

18. Narita M et al. Immunoglobulin G avidity testing in serum and cerebrospinal fluid for analysis of measles virus infection. *Clinical and diagnostic laboratory immunology*, 1996, 3:211–5.

19. Ward KN et al. Avidity of IgG antibodies to human herpesvirus-6 distinguishes

primary from recurrent infection in organ transplant recipients and excludes cross-reactivity with other herpesviruses. *Journal of medical virology*, 1993, 39:44–9.

20. Hedman K et al. Maturation of immunoglobulin G avidity after rubella vaccination studied by an enzyme linked immunosorbent assay (avidity-ELISA) and by haemolysis typing. *Journal of medical virology*, 1989, 27:293–8.

21. Yohannes Y, Hoddinott J. *Classification and regression trees: an introduction*. Washington DC, International Food Policy Research Institute, 1999.

22. Greenland S, Robins JM. Estimation of a common parameter from sparse follow-up data. *Biometrics*, 1985, 41:55–68.

23. Measles eradication: recommendations from a meeting cosponsored by the World Health Organization, the Pan American Health Organization, and CDC. *Morbidity and mortality weekly report. Recommendations and reports*, 1997, 46:RR-11.

24. Hamkar R et al. Epidemiologic features of viral exanthematous diseases in Iran. In: *Proceedings of the Second Iranian Congress on Virology, February 2004*. Tehran, Islamic Republic of Iran, Iranian Society for Virology, 2004.

25. Mossong J, Muller CP. Modelling measles re-emergence as a result of waning of immunity in vaccinated populations. *Vaccine*, 2003, 21:4597–603.

26. Erdman DD et al. Immunoglobulin M antibody response to measles virus flowing primary and secondary vaccination and natural virus infection. *Journal of medical virology*, 1993, 1:44–8.

27. Helfand RF et al. Diagnosis of measles with an IgM capture EIA: the optimal timing of specimen collection after rash onset. *Journal of infectious diseases*, 1997, 175:195–9.

28. Hidaka Y et al. Serological and clinical characteristics of measles vaccine failure in Japan. *Scandinavian journal of infectious diseases*, 1994, 26:725–30.