Vaccine 34 (2016) 4166–4171

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Mumps-specific cross-neutralization by MMR vaccine-induced antibodies predicts protection against mumps virus infection

Sigrid Gouma [a,b,*], Hinke I. ten Hulscher [a], Tessa M. Schurink-van 't Klooster [a], Hester E. de Melker [a], Greet J. Boland [c], Patricia Kaaijk [a], Cécile A.C.M. van Els [a], Marion P.G. Koopmans [a,b], Rob S. van Binnendijk [a]

[a] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[b] Department of Viroscience, Erasmus University Medical Centre, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands
[c] Department of Medical Microbiology, University Medical Centre Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 28 April 2016
Received in revised form 7 June 2016
Accepted 20 June 2016
Available online 29 June 2016

Keywords:
Strain-specific neutralization
IgG antibodies
MMR vaccination
Mumps virus
Correlate of protection
Asymptomatic infection

ABSTRACT

Background: Similar to other recent mumps genotype G outbreaks worldwide, most mumps patients during the recent mumps genotype G outbreaks in the Netherlands had received 2 doses of measles, mumps and rubella (MMR) vaccine during childhood. Here, we investigate the capacity of vaccine-induced antibodies to neutralize wild type mumps virus strains, including mumps virus genotype G.
Methods: In this study, we tested 105 pre-outbreak serum samples from students who had received 2 MMR vaccine doses and who had no mumps virus infection ($n = 76$), symptomatic mumps virus infection ($n = 10$) or asymptomatic mumps virus infection ($n = 19$) during the mumps outbreaks. In all samples, mumps-specific IgG concentrations were measured by multiplex immunoassay and neutralization titers were measured against the Jeryl Lynn vaccine strain and against wild type genotype G and genotype D mumps virus strains.
Results: The correlation between mumps-specific IgG concentrations and neutralization titers against Jeryl Lynn was poor, which suggests that IgG concentrations do not adequately represent immunological protection against mumps virus infection by antibody neutralization. Pre-outbreak neutralization titers in infected persons were significantly lower against genotype G than against the vaccine strain. Furthermore, antibody neutralization of wild type mumps virus genotype G and genotype D was significantly reduced in pre-outbreak samples from infected persons as compared with non-infected persons. No statistically significant difference was found for the vaccine strain. The sensitivity/specificity ratio was largest for neutralization of the genotype G strain as compared with the genotype D strain and the vaccine strain.
Conclusions: The reduced neutralization of wild type mumps virus strains in MMR vaccinated persons prior to infection indicates that pre-outbreak mumps virus neutralization is partly strain-specific and that neutralization differs between infected and non-infected persons. Therefore, we recommend the use of wild type mumps virus neutralization assays as preferred tool for surveillance of protection against mumps virus infection.

1. Introduction

At the end of 2009, large mumps outbreaks started in the Netherlands and continued for 3 years, followed by some years with smaller and more local mumps outbreaks [1,2]. Most of the patients were young adults who had received 2 measles, mumps, and rubella (MMR) vaccinations during childhood at 14 months and 9 years of age, according to the Dutch national immunization program [3,4]. Similar to other recent mumps outbreaks among MMR vaccinated persons worldwide, the mumps outbreaks in the Netherlands were dominated by mumps genotype G virus strains, whereas the Jeryl Lynn mumps strain of the MMR vaccine belongs to genotype A. It has been shown previously that vaccinated persons develop sufficient neutralizing antibodies against wild type mumps genotype G virus, although the neutralization capacity is lower against the

* Corresponding author at: Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands.
E-mail addresses: sigrid.gouma@rivm.nl (S. Gouma), hinke.ten.hulscher@rivm.nl (H.I. ten Hulscher), tessa.schurink@rivm.nl (T.M. Schurink-van 't Klooster), hester.de.melker@rivm.nl (H.E. de Melker), G.J.Boland@umcutrecht.nl (G.J. Boland), patricia.kaaijk@rivm.nl (P. Kaaijk), cecile.van.els@rivm.nl (C.A.C.M. van Els), m.koopmans@erasmusmc.nl (M.P.G. Koopmans), rob.van.binnendijk@rivm.nl (R.S. van Binnendijk).

http://dx.doi.org/10.1016/j.vaccine.2016.06.063
0264-410X/

wild type strain as compared with the vaccine strain [5,6]. However, no correlate of protection has been defined so far that distinguishes MMR vaccinated persons with sufficient immunological protection from those who are not protected against mumps virus infection.

Here, we aimed to investigate the capacity of vaccine-induced antibodies to neutralize various mumps virus strains and to establish a correlate of protection based on pre-outbreak neutralizing antibody titers. Pre-outbreak serum samples were selected from MMR vaccinated students who were infected with mumps virus during the outbreak and these samples were compared with samples from non-infected students. The correlation between mumps-specific IgG concentrations and functional antibodies against the Jeryl Lynn vaccine strain was determined. Furthermore, we studied strain-specific neutralization between the Jeryl Lynn vaccine strain, mumps virus genotype G, which circulated in the Netherlands during the recent mumps outbreaks among vaccinated persons, and mumps virus genotype D, which caused a mumps outbreak among unvaccinated children in 2007–2009 in the Netherlands [7]. We investigated pre-outbreak samples from both persons with symptomatic and asymptomatic infections to determine if reduced neutralization of wild type strains affects the occurrence of clinical mumps in mumps virus infected persons.

2. Methods

2.1. Study subjects and pre-outbreak serum samples

All pre-outbreak sera used in this study were samples from a medically ethically approved serological study previously described (NL38042.041.11) [8]. This retrospective study was performed among students from the city of Utrecht in 2012. Besides a self-sampled dried blot spot sample and a filled out questionnaire concerning MMR vaccination history, risk factors and mumps symptoms, a serum sample was retrospectively obtained from each student. In addition, MMR vaccination history was verified for 80.5% of the students from the data recorded in the nationwide vaccination registration system (Praeventis) [8]. In total, 619 pre-outbreak serum samples collected between 2007 and 2010 were obtained and included for the initial analysis to identify mumps virus infections based on mumps-specific antibody concentrations [8]. For this study, a selection was made consisting of 105 pre-outbreak serum samples from persons who had received 2 MMR doses and who had symptomatic mumps virus infection, asymptomatic mumps virus infection or no mumps virus infection during the mumps outbreaks that followed. The serological criteria for mumps virus infection were a fourfold increase in IgG concentration or a single-point cutoff at 1500 RU/ml [8]. Persons with symptomatic mumps virus infection fulfilled at least one of these serological criteria and indicated in the questionnaire that they developed mumps symptoms during the period 2009–2012 ($n = 10$). Persons with asymptomatic mumps virus infection fulfilled also at least one of the serological criteria, but they did not report any mumps symptoms in the questionnaire ($n = 19$). Non-infected persons did not fulfill any of the serological criteria for mumps virus infection and did not report mumps symptoms ($n = 76$). The 105 pre-outbreak serum samples selected for this study included all available samples from infected persons that fulfilled the criteria in this study and a selection of the samples from non-infected persons. The geometric mean IgG concentrations in the selected pre-outbreak samples from persons without a mumps virus infection did not differ from the total cohort from the initial study (192 RU/ml versus 171 RU/ml; $p = 0.618$) [8].

2.2. Cell culturing and mumps virus isolation

Vero cells (Monkey African Green Kidney, ECACC) were cultured at 37 °C in 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal calf serum (FCS), penicillin, streptomycin, and L-glutamine. For inoculation of the Jeryl Lynn mumps virus strain, DMEM supplemented with 2% FCS, penicillin, streptomycin, and L-glutamine was used. The same medium composition was used for isolation of wild type mumps virus strains, with the addition of nystatin. Virus culturing was performed at 36 °C in 5% CO_2. Mumps virus Jeryl Lynn strain was seeded on Vero cells and passaged 2 times before harvesting. Mumps virus strains belonging to genotype G (MuVi/Utrecht.NLD/40.10) and genotype D (MuVi/Sint Philipsland.NLD/02.08) were isolated from laboratory-confirmed mumps virus-positive throat swabs and were passaged 2 times and 4 times respectively before harvesting. Virus stocks were stored at −80 °C until use. Aliquots were made from all virus stocks to avoid multiple freeze–thaw cycles.

2.3. Multiplex IgG immunoassay

Mumps-specific IgG concentrations were measured with a fluorescent bead-based multiplex immunoassay (MIA) using Luminex technology as described previously [9]. Purified Jeryl Lynn antigen was coupled to carboxylated beads for detection of mumps-specific IgG. Samples were 1:200 diluted in assay buffer (phosphate buffered saline (PBS) containing 0.1% Tween-20 and 3% bovine serum albumin). On each plate, the WHO International Standard Anti Rubella Immunoglobulin RUBI-1-94 (The National Institute for Biological Standards and Control), controls and blanks were included. Antibody concentrations were expressed in local (RIVM) units per milliliter (RU/ml) and were based on the fluorescent intensity of the reference serum curve RUBI-1-94, which has a mumps-specific IgG concentration of 4384.512 RU/ml and was selected as alternative serological standard for mumps to enable comparison and bridging of our results to other studies [8]. When the 1:200 sample dilution fell outside the range of the reference serum curve, further dilutions up to 1:50,000 were used for analysis.

2.4. Focus-reduction neutralization test (FRNT)

Neutralization by vaccine-induced antibodies was tested by FRNT, partly based on the protocol described by Vaidya et al. [10]. Neutralization tests were performed in 96-wells plates. Four-fold dilutions were made in DMEM supplemented with 2% FCS, penicillin, streptomycin, and L-glutamine. Viruses and samples were mixed and incubated for 2 h at 37 °C. Medium was removed from pre-cultured Vero cells and 50 µl of virus mixture was added to each well. Plates were incubated for 4 h at 36 °C, before the mixture was removed from the Vero cells and 200 µl of 0.8% carboxymethylcellulose in DMEM was added to each well. Plates were incubated for 40 h at 36 °C with 5% CO_2 before they were washed with PBS and thereafter fixed with a mixture of acetone and methanol (2:3). After 10 min, plates were washed with ice cold PBS, and then incubated with block buffer (PBS containing 1% bovine serum albumin) for 30 min at 36 °C. Anti-mumps nucleoprotein antibody (7B10, Abcam) was diluted in block buffer (1:3000) and 100 µl was added to each well. After incubation for one hour at 36 °C, plates were washed 3 times with PBS containing 0.1% Tween-20 (PBST) before 100 µl of goat-anti-mouse IgG-HRP (DAKO) diluted in block buffer (1:2000) was added to each well and plates were incubated for one hour at 36 °C. Plates were washed 3 times with PBST and wells were stained with 50 µl of True Blue peroxidase substrate (Kirkegaard & Perry Laboratories). The numbers of plaques were counted and the 50% neutralizing dose (ND_{50}) of each sample was calculated with the Kärber for-

mula, using the serum dilution factor as calculated from the virus mixture [10].

The WHO international standard RUBI-1-94 was used as positive control in each assay run. For a valid assay, the titer of RUBI-1-94 was required to be within 2 standard deviations of its cumulative historical mean value. Furthermore, assays were only valid if the mean plaque number of the virus controls was in the range of 20–80 and cell controls had no plaques. If assays did not meet the validation criteria, samples were retested. Only samples with at least two valid results per virus strain in separate runs were used for analyses. To correct for inter-assay differences, we normalized the ND_{50} values by multiplying the raw ND_{50} titers by the RUBI-1-94 factor, which was defined as the cumulative historical mean value of RUBI-1-94 divided by the measured ND_{50} of RUBI-1-94.

2.5. Statistical analysis

Geometric mean (with 95% confidence interval [CI]) IgG concentrations and neutralization titers were compared between infected and non-infected persons using the Kolmogorov–Smirnov test. Correlations between IgG concentrations and neutralization titers were computed using nonlinear regression with the Spearman correlation coefficient. For comparison of neutralization titers against different mumps virus strains, the Friedman test was used. With a receiver operator characteristics (ROC) analysis, the area under the curve (AUC) with 95% confidence limits was calculated for mumps-specific IgG concentrations and neutralizing antibodies against the different mumps virus strains to determine a correlate of immune protection. The DeLong test was used for comparison of AUCs, using the genotype G neutralization titer as Ref. [11]. All analyses were performed using SPSS version 22, SAS version 9 and GraphPad Prism version 6. *P* values <0.05 were considered as statistically significant.

3. Results

3.1. Mumps-specific IgG concentrations

Since mumps-specific IgG concentrations are widely used to define levels of mumps immunity, mumps-specific IgG concentrations were studied in pre-outbreak serum samples from infected and non-infected persons. Mumps-specific IgG concentrations were measured by MIA using purified Jeryl Lynn as antigen and these results were compared with neutralization titers against the same mumps virus Jeryl Lynn strain. In this way, we could study the correlation between mumps-specific IgG concentrations and neutralization titers.

Mumps-specific IgG concentrations were significantly lower in pre-outbreak samples from infected persons than in samples from non-infected persons (122 RU/ml (86–173 RU/ml) versus 192 RU/ml (161–228 RU/ml); $p = 0.008$). The Jeryl Lynn-specific neutralization titer in pre-outbreak samples from infected and non-infected persons was ND_{50} 67 (47–95) and ND_{50} 115 (87–152), respectively ($p = 0.078$). The correlation between IgG concentrations and neutralization titers against Jeryl Lynn in pre-outbreak samples was poor for both the infected and non-infected persons ($r^2 = 0.017$ and $r^2 = 0.165$, respectively; Fig. 1). There was no difference in mumps-specific IgG concentrations and neutralization titers between symptomatic and asymptomatic mumps virus infections.

3.2. Strain-specific neutralization in pre-outbreak samples

To investigate if antigenic differences between the vaccine strain and wild type strains resulted in reduced neutralization of the wild type strains, we measured virus neutralization in pre-outbreak serum samples against the Jeryl Lynn vaccine strain as well as against wild type genotype G and genotype D strains. In pre-outbreak samples from persons without a mumps virus infection, neutralization titers against the different mumps virus strains did not significantly differ ($p > 0.105$; Fig. 2A). For infected persons pre-outbreak neutralization titers against genotype G were significantly lower than neutralization titers against Jeryl Lynn ($p = 0.009$; Figs. 2A and 3). When the mumps virus infected group was further divided into symptomatic and asymptomatic mumps virus infections, only in the asymptomatic group neutralization titers against genotype G were significantly lower than neutralization titers against Jeryl Lynn ($p = 0.036$; Fig. 2B). The lack of significant results in the symptomatic group may be due to the low number of symptomatic infections in our study cohort.

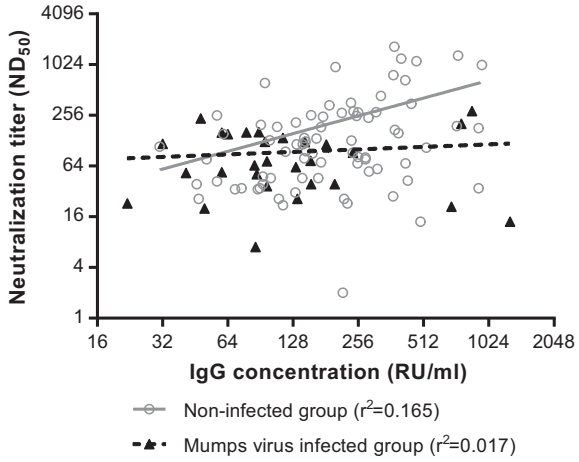

Fig. 1. Correlation between mumps-specific IgG concentrations and neutralization titers against Jeryl Lynn in pre-outbreak samples from vaccinated students ($n = 105$). Lines represent the best fit lines with nonlinear regression.

3.3. Correlate of protection

To establish a correlate of protection based on mumps-specific neutralizing antibodies, pre-outbreak neutralization titers were compared between infected and non-infected persons. The difference in pre-outbreak neutralization titers against Jeryl Lynn between infected and non-infected persons was not significant ($p = 0.078$; Fig. 2A). However, neutralization titers against the wild type mumps virus genotype G and genotype D strains were significantly lower in infected persons than in non-infected persons ($p = 0.011$ and $p = 0.022$, respectively; Fig. 2A), which suggests that the capacity of vaccine-induced antibodies to neutralize wild type strains is a more appropriate marker for protection. The difference in neutralization titers between symptomatic and asymptomatic infections was not significant (Fig. 2B).

A ROC analysis with the 3 different mumps virus strains to compare sensitivity/specificity ratios resulted in an AUC of 0.703 (0.595–0.812) for genotype G, which is larger than the AUCs for Jeryl Lynn (0.628 (0.516–0.741); $p = 0.071$) and for genotype D (0.638 (0.526–0.750); $p = 0.090$), although the differences were not statistically significant (Fig. 4). A ROC analysis based on the IgG concentrations resulted in an AUC of 0.689 (0.564–0.813), which is also not significantly different from the AUC for genotype G ($p = 0.841$; Fig. 4). No cutoff could be established that separated all mumps virus infections from all non-infections. For mumps-specific IgG concentrations, the cutoff at 243 RU/ml, as published previously, resulted in a sensitivity of 86.21% and a specificity of

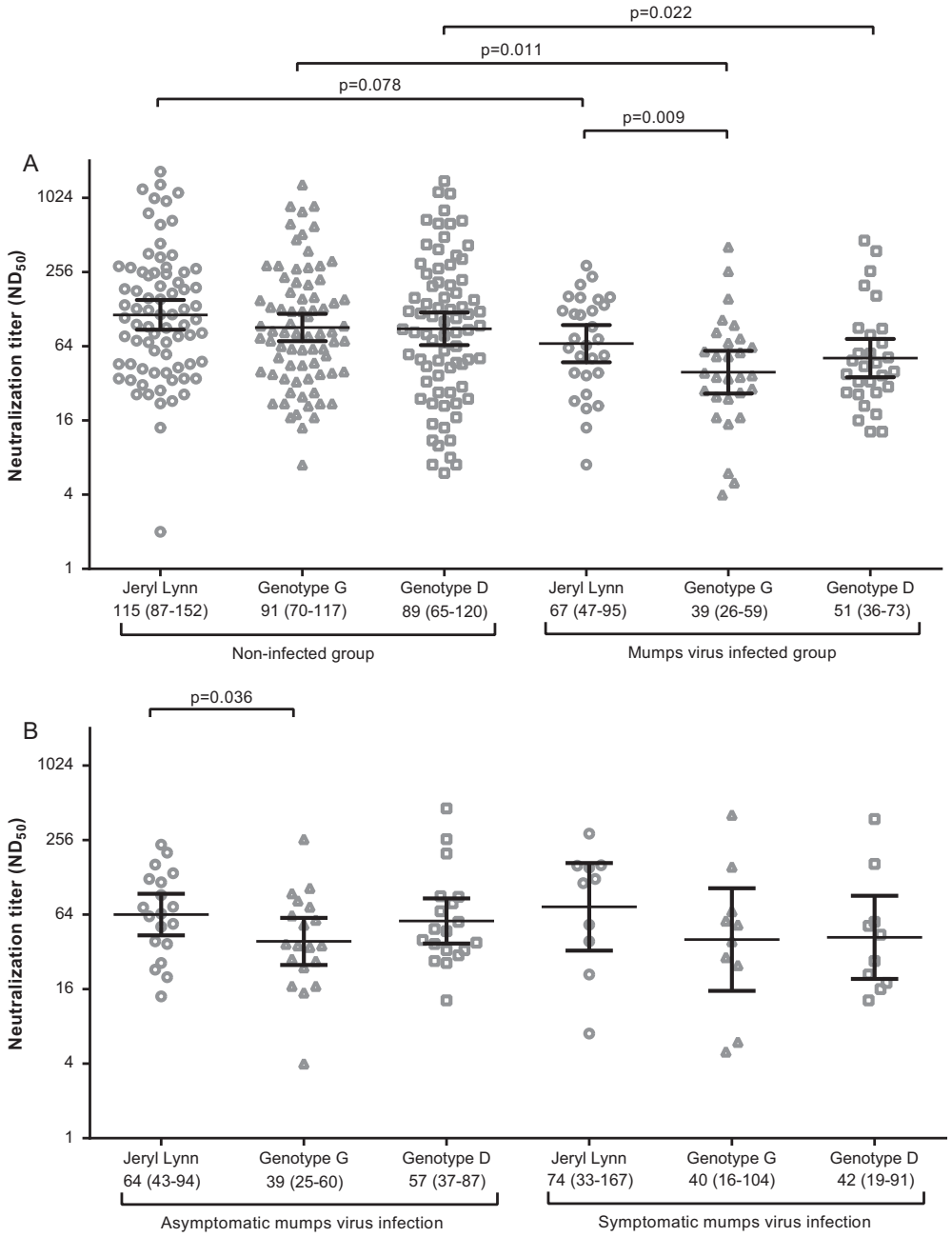

Fig. 2. Neutralization titers against the different mumps virus strains. *P* values <0.10 are shown above the graphs. Geometric mean neutralization titers with 95% confidence interval are shown on the X axes. (A) Neutralization titers in persons with or without a mumps virus infection. (B) Neutralization titers in mumps virus infected persons, further differentiated into symptomatic and asymptomatic mumps virus infections.

39.47% when applied to the pre-outbreak samples from this study [8]. When this sensitivity of 86.21% was used to calculate a cutoff for the neutralization titers, the cutoff for Jeryl Lynn-specific neutralization was at ND_{50} 167 (89.66% sensitivity and 38.16% specificity), which was comparable with the cutoff for genotype D-specific neutralization (ND_{50} 169 with 86.21% sensitivity and 30.26% specificity). The cutoff value for genotype G-specific neutralization was lower (ND_{50} 97 with 86.21% sensitivity and 43.42% specificity). These data indicate that the sensitivity/specificity ratio was most optimal for neutralizing antibody titers against the mumps virus genotype G strain, although differences were not statistically significant. Furthermore, the specificity at these cutoffs was highest for genotype G-specific neutralization.

4. Discussion

In pre-outbreak serum samples from mumps virus infected persons who had received 2 MMR doses, neutralization titers against mumps virus genotype G were lower than against the Jeryl Lynn strain, which suggests that neutralization by vaccine-induced antibodies is partly strain-specific. This difference in neutralization between the vaccine strain and a wild type mumps virus genotype G strain was previously described by others [5,6]. Contrary to those previous studies, we only observed this difference in pre-outbreak serum samples from infected persons. Furthermore, in our study only neutralization titers against the wild type genotype G and genotype D mumps viruses differed between infected and non-

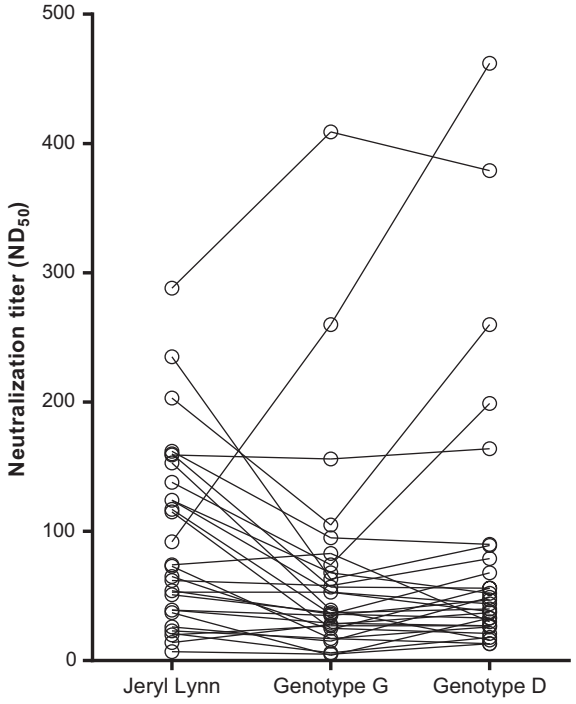

Fig. 3. Paired neutralization titers against the different mumps virus strains in pre-outbreak samples from infected persons. Neutralization titers were significantly lower against the wild type genotype G strain than against the Jeryl Lynn vaccine strain ($p = 0.009$). There were no significant differences between the wild type genotype D strain and any of the other strains.

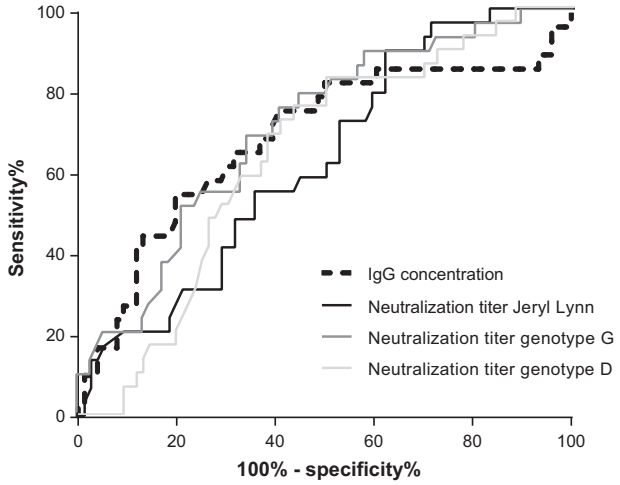

Fig. 4. Receiver operator characteristics analysis based on IgG concentrations and neutralization titers against Jeryl Lynn, genotype G and genotype D mumps virus strains.

infected persons, whereas in one of the previous studies the neutralization titers against Jeryl Lynn were lower for infected persons than for non-infected persons [5].

In our study, both symptomatic and asymptomatic mumps virus infections were identified using reported symptoms as well as mumps-specific IgG concentrations as criteria for mumps virus infection. In this way, asymptomatic mumps virus infections could be identified and these were excluded from the non-infected group. Inclusion of asymptomatically infected persons in the non-infected group could affect the results. This might explain the difference in results between our study and previous studies in which pre-outbreak neutralization titers were compared between mumps patients and non-patients. In spite of the use of mumps-specific IgG concentrations as criteria for mumps virus infection, it cannot be excluded that some students had been exposed to mumps earlier. The high neutralization titers against the wild type viruses in 2 pre-outbreak samples from infected students suggest that these persons may have been previously infected with mumps virus, although surveillance data showed no evidence for previous mumps virus infections in our study cohort (Fig. 3).

The poor correlation between mumps-specific IgG concentrations and neutralization titers observed in our study confirmed results from other studies [10,12–14]. The MIA that was used to measure the mumps-specific IgG concentrations is based on purified Jeryl Lynn virus and measures the total amount of mumps-specific IgG present in the sample. In contrast, the neutralization assay was used to measure virus neutralizing antibodies, which may be an important immunological mechanism in protection against mumps virus infection [15,16]. ROC analysis showed that the sensitivity/specificity ratio is largest for genotype G-specific neutralization titers. Although no cutoff value for pre-outbreak neutralization titers could be identified that separates all infected persons from non-infected persons, our data suggest that genotype G-specific neutralization is a more adequate serological marker for protection against mumps virus infection, as most of the recent mumps outbreaks among MMR vaccinated persons were caused by mumps virus genotype G strains.

The results from this study suggest that antigenic differences between the mumps virus vaccine strain and wild type strains affect mumps-specific humoral immune responses in MMR vaccinated persons. Two mumps virus membrane proteins that play a major role in virus neutralization are the fusion (F) protein and the haemagglutinin-neuraminidase (HN) protein [17–19]. As was hypothesized previously, the difference in neutralization between Jeryl Lynn and the wild type strains may result from antigenic differences in the neutralizing epitopes of the F and HN proteins [6]. Because of the potential biological relevance of mumps virus neutralization and the difference in strain-specific neutralization between infected and non-infected persons, we recommend the use of wild type mumps virus neutralization assays as preferred tool for surveillance of protection against mumps virus infection.

Authors' contribution

SG, HdM, MK and RvB designed the study. SG wrote the initial draft of the manuscript. TS, HdM, GB, PK, CvE, MK and RvB revised the manuscript critically with important conceptual contributions. GB was involved in recruitment of the participants and sample collection. SG and HtH collected the data. SG and TS conducted the statistical analysis. All authors approved the final version of the manuscript.

Conflicts of interest

None declared.

Acknowledgements

We acknowledge the students for participating in this study. Furthermore, we thank Daphne Gijselaar, Fokko Meindertsma, and Maaike Suuring for technical assistance.

References

[1] Gouma S, Sane J, Gijselaar D, Cremer J, Hahné S, Koopmans M, et al. Two major mumps genotype G variants dominated recent mumps outbreaks in the Netherlands (2009–2012). J Gen Virol 2014;95:1074–82.
[2] Gouma S, Cremer J, Parkkali S, Veldhuijzen I, van Binnendijk RS, Koopmans MPG. Mumps virus F gene and HN gene sequencing as a molecular tool to study mumps virus transmission, Genet Evol, submitted for publication.

[3] Whelan J, van Binnendijk R, Greenland K, Fanoy E, Khargi M, Yap K, et al. Ongoing mumps outbreak in a student population with high vaccination coverage, Netherlands, 2010. Eurosurveillance 2010;15:11–4.
[4] Sane J, Gouma S, Koopmans M, De Melker H, Swaan C, Van Binnendijk R, et al. Epidemic of mumps among vaccinated persons, the Netherlands, 2009–2012. Emerg Infect Dis 2014;20:643–8.
[5] Cortese MM, Barskey AE, Tegtmeier GE, Zhang C, Ngo L, Kyaw MH, et al. Mumps antibody levels among students before a mumps outbreak: in search of a correlate of immunity. J Infect Dis 2011;204:1413–22.
[6] Rubin SA, Qi L, Audet SA, Sullivan B, Carbone KM, Bellini WJ, et al. Antibody induced by immunization with the Jeryl Lynn mumps vaccine strain effectively neutralizes a heterologous wild-type mumps virus associated with a large outbreak. J Infect Dis 2008;198:508–15.
[7] Wielders CC, van Binnendijk RS, Snijders BE, Tipples GA, Cremer J, Fanoy E, et al. Mumps epidemic in orthodox religious low-vaccination communities in the Netherlands and Canada, 2007 to 2009. Eurosurveillance 2011;16:19989.
[8] Gouma S, Schurink-van't Klooster TM, de Melker HE, Kerkhof J, Smits GP, Hahné SJM, et al. Mumps serum antibody levels before and after an outbreak to assess infection and immunity in vaccinated students. Open Forum Infect Dis 2014;1. ofu101.
[9] Smits GP, Van Gageldonk PG, Schouls LM, Van Der Klis FRM, Berbers GAM. Development of a bead-based multiplex immunoassay for simultaneous quantitative detection of IgG serum antibodies against measles, mumps, rubella, and varicella-zoster virus. Clin Vaccine Immunol 2012;19:396–400.
[10] Vaidya SR, Brown DWG, Jin L, Samuel D, Andrews N, Brown KE. Development of a focus reduction neutralization test (FRNT) for detection of mumps virus neutralizing antibodies. J Virol Methods 2010;163:153–6.
[11] DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–45.
[12] Date AA, Kyaw MH, Rue AM, Klahn J, Obrecht L, Krohn T, et al. Long-term persistence of mumps antibody after receipt of 2 measles-mumps-rubella (MMR) vaccinations and antibody response after a third MMR vaccination among a university population. J Infect Dis 2008;197:1662–8.
[13] Matsubara K, Iwata S, Nakayama T. Antibodies against mumps virus component proteins. J Infect Chemother 2012;18:466–71.
[14] Latner DR, McGrew M, Williams NJ, Sowers SB, Bellini WJ, Hickman CJ. Estimates of mumps seroprevalence may be influenced by antibody specificity and serologic method. Clin Vaccine Immunol 2014;21:286–97.
[15] Mauldin J, Carbone K, Hsu H, Yolken R, Rubin S. Mumps virus-specific antibody titers from pre-vaccine era sera: comparison of the plaque reduction neutralization assay and enzyme immunoassays. J Clin Microbiol 2005;43:4847–51.
[16] Vaidya S, Dvivedi G, Jadhav S. Cross-neutralization between three mumps viruses & mapping of haemagglutinin-neuraminidase (HN) epitopes. Indian J Med Res 2016;143:37–42.
[17] Cusi MG, Fischer S, Sedlmeier R, Valassina M, Valensin PE, Donati M, et al. Localization of a new neutralizing epitope on the mumps virus hemagglutinin-neuraminidase protein. Virus Res 2001;74:133–7.
[18] Kulkarni-Kale U, Ojha J, Manjari GS, Deobagkar DD, Mallya AD, Dhere RM, et al. Mapping antigenic diversity and strain specificity of mumps virus: a bioinformatics approach. Virology 2007;359:436–46.
[19] Santak M, Orvell C, Ko uti Gulija T. Identification of conformational neutralization sites on the fusion protein of mumps virus. J Gen Virol 2015;96:982–90.