Sensitivity and specificity of immunoglobulin G titer for the diagnosis of mumps virus in infected patients depending on vaccination status

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The aim of the study was to evaluate the usefulness of serological detection of mumps IgM and titration of IgG in patients with acute parotitis according to their vaccination status. The detection of mumps virus RNA in saliva by RT-PCR was used as reference. 116 patients (109 of them previously vaccinated) with mumps RT-PCR-negative results and 21 (19 vaccinated) with mumps RT-PCR-positive results were studied. Mumps-specific IgM and IgG were assayed by EIA (Enzygnost, Dade Behring, Germany). IgM results were expressed as positive or negative. For IgG, several cut-offs were calculated using receiver operating characteristic (ROC) curves. Seven RT-PCR-positive and five RT-PCR-negative patients showed IgM-positive results (sensitivity 33.3% and specificity 95.7%). Among vaccinated patients, the sensitivity and specificity of IgM were 26.3% (5/19) and 99.1% (108/109). For IgG, a titer of 5,000 in all the patients showed a sensitivity of 76.2% (16/21) and a specificity of 83.6% (97/116). In vaccinated patients, the corresponding figures for this cut-off were 84.2% (16/19) and 83.5% (91/109), respectively. Although IgM detection against mumps is highly specific, its sensitivity is very low in immunized subjects. In this group, the titration of IgG could serve as an additional diagnostic tool.

Key words: Mumps; IgM; IgG; serology; vaccine.

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An increase in the notification of cases and outbreaks of mumps virus infections has been observed in Spain (1, 2) and other European countries (3) during recent years. Low vaccination coverage, inadequate vaccination scheduling, and combinations of primary and secondary vaccine failure have been proposed as possible causes for the reemergence of this disease (4).

Despite the increase in the incidence of mumps, only a small proportion of suspected cases are confirmed by specific IgM determination. Some virological and immunological features of this infection have made serological diagnosis in vaccinated populations cumbersome and inconclusive:

(a) Immunogenicity against mumps is not
necessarily an accurate marker of vaccine effectiveness (5); vaccination with live attenuated mumps virus induces relatively low levels of neutralizing antibodies compared with natural infection, and some vaccinated subjects may have EIA-detectable but nonprotective antibodies (6); immunity may be incomplete, making reinfections possible (7).

(b) Evolution and adaptation of a circulating mumps virus variant in the community can lead to the emergence of genetically distinct viral strains (8); new mumps virus variants could present reduced cross-neutralization capacity in the presence of current vaccine strains (9); neutralizing antibodies against a specific strain may not be protective for other heterologous genotypes (10).

In Spain, where good vaccination coverage with MMR is maintained (11), the role of secondary vaccine failure could be an important factor in the appearance of outbreaks (1). The Rubini strain used in the MMR vaccine during the 1990s (2) has been considered less immunogenic and effective (12–14) than other vaccine strains. On the other hand, no vaccines ensure absolute immunity (1, 5).

In previously immunized patients suffering from mumps infection, the serological reaction is a typical secondary immune response, with a peak of IgG and little or no rise in IgM. Due to the difficulties of laboratory confirmation using classic serological criteria, detection of very high titers of specific IgG has been used for diagnosis in other immunopreventable diseases (15). The objective of this study was to evaluate the diagnostic achievement of IgM determination and IgG titration against mumps, comparing the results obtained with direct detection by RT-PCR, in patients with acute disease with or without a known history of mumps vaccination.

MATERIALS AND METHODS

137 patients with compatible symptoms of acute parotitis were studied. Age, time elapsed from onset of disease and number of doses of measles-mumps-rubella (MMR) vaccine previously administered were recorded. Serum and saliva samples were collected simultaneously from all subjects.

Detection of mumps viral RNA in saliva, performed following a RT-PCR method previously described (16), was accepted as the reference for diagnosis. Patients were clustered according to the result of the RT-PCR. 116 (109 of them previously vaccinated) showed RT-PCR-negative results and 21 (19 previously vaccinated) were RT-PCR positive. In all patients, adenovirus, enterovirus and herpesvirus (other viruses potentially causing parotitis) were ruled out by molecular methods (17, 18).

Serum samples were assayed for determination of specific IgM and IgG against mumps virus using EIA techniques (Enzygnost; Dade Berhing, Germany). Both qualitative mumps-specific IgM and IgG, and quantitative specific IgG determinations (estimated by mean of \( \alpha \)-method) were run following the manufacturer’s instructions. For calculation of geometric means of the titer (GMT) of IgG, an arbitrary value, corresponding to half the cut-off (115 titer), was assigned to seronegative subjects. The relation between IgG-antibody logarithmic titers according to RT-PCR results and the time after onset of symptoms was determined by calculating the quadratic coefficient of correlation of Pearson (\( R^2 \)). Sensitivity and specificity of IgM determination and IgG (best cut-off values for diagnosis in terms of titers), according to vaccination status, were taken from RT-PCR results for mumps RNA virus to be the reference standard. The best cut-off points for IgG titers were determined using receiver operating characteristic (ROC) curve analysis. The study of qualitative variables was carried out by proportion comparisons (using \( \chi^2 \), \( \chi^2 \) with Yates correction, or \( \chi^2 \) two-tailed Fisher’s exact test when required). Differences among means of quantitative variables, such as age, time elapsed since onset of the disease, number of vaccine doses, and IgG GMT, were established by analyzing the variance. Values of \( p<0.05 \), calculated by Epi Info 6.0, were considered significant. Means and proportions (sensitivity and specificity) were calculated with 95% confidence intervals (CI 95%).

RESULTS

Mumps RT-PCR-positive and -negative patients were comparable with respect to age (mean age 9.1 [CI 95% 5.6–12.6] versus 8.0 [CI 95% 6.8–9.2]), number of vaccine doses administered (mean of doses 1.2 [CI 95% 0.9–1.5] versus 1.4 [CI 95% 1.3–1.5]), and days elapsed since onset of disease (mean of days since onset 2.9 [CI 95% 1.8–4.0] versus 4.4 [CI 95% 3.5–5.3]). During the first 6 days after symptoms, a positive result was obtained in 20/21 (95.2%) of RT-PCR-positive saliva samples, and in 98/116 (84.5%) of RT-PCR-negative saliva samples (\( p<0.05 \); \( \chi^2 \) two-tailed Fisher’s exact test).

The IgG antibody logarithmic titers in relation to time after onset of symptoms, accord-
Fig. 1. IgG-antibody logarithmic titers in relation to time after onset of symptoms, according to RT-PCR results in total (A) and in previously vaccinated patients (B).

Table 1. Seropositivity levels and geometric means of IgG titers against mumps in mumps RT-PCR-positive and -negative patients

<table>
<thead>
<tr>
<th></th>
<th>IgG positivity</th>
<th>P value</th>
<th>GMT (CI95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total mumps RT-PCR negative patients</td>
<td>86.2% (100/116)</td>
<td>&gt;0.05*</td>
<td>3.0 (2.9–3.1)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Total mumps RT-PCR positive patients</td>
<td>95.2% (20/21)</td>
<td>3.9 (3.6–4.2)</td>
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</tr>
<tr>
<td>Vaccinated mumps RT-PCR negative patients</td>
<td>87.2% (95/109)</td>
<td>&gt;0.05*</td>
<td>3.0 (2.9–3.1)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Vaccinated mumps RT-PCR positive patients</td>
<td>94.7% (18/19)</td>
<td>4.0 (3.7–4.3)</td>
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*χ² (Yates correction).
**F statistic (analysis of variance).
IgG. Seven RT-PCR-positive and five RT-PCR-negative patients were IgM positive (sensitivity 33.3% and specificity 95.7%). Among vaccinated patients, sensitivity and specificity were 26.3% (5/19) and 99.1% (108/109). Specificity levels of the IgG considered cut-offs were significantly lower (p<0.01) than specificity levels for IgM detection. Conversely, the sensitivity for the IgG titers ≥2,700 and ≥5,000 in the total group of patients studied reached significantly higher levels (p<0.01) compared to the sensitivity of IgM detection. In vaccinated patients, sensitivities obtained with both established cut-offs (IgG≥5,000 and IgG≥5,400) were also significantly higher (p<0.01) than the sensitivity of IgM detection.

The nine non-vaccinated patients were five adults, three children under 16 months, and one child aged 6 years. Among these, two were mumps RT-PCR positive. Both were IgM positive and showed IgG titers ≥2,700. Among the seven RT-PCR-negative non-vaccinated patients, four IgM-positive results were observed, and two of these also showed IgG titers ≥2,700.

The distribution of the 12 positive IgM results according to the patient’s vaccination status (regardless of RT-PCR results) was 6 positive results in 9 of the non-vaccinated (66.7%) and in 6 of the 128 non-vaccinated (4.7%). This difference was significant (p<0.001 two-tailed Fisher’s exact tests).

Considering only the 125 IgM-negative patients (irrespective of vaccination antecedents) the IgG cut-off titers of ≥2,700, ≥5,000 and ≥5,500 give percentages of sensitivity and specificity, respectively, of 92.9 (13/14), 92.9 (13/14) and 85.7 (12/14), and 74.8 (83/111), 84.7 (94/111) and 86.5 (96/111).

### TABLE 2. Sensitivity and specificity of IgM and IgG cut-off values

<table>
<thead>
<tr>
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<th>Sensitivity (CI95%)</th>
<th>Specificity (CI95%)</th>
<th>Association with RT-PCR</th>
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<tbody>
<tr>
<td><strong>Total of patients (n=137)</strong></td>
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<tr>
<td>IgM</td>
<td>33.3 (15.5–56.9)</td>
<td>95.7 (89.7–98.4)</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>IgG titer ≥5,000</td>
<td>76.2 (52.4–90.9)</td>
<td>83.6 (75.3–89.6)</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>IgG titer ≥2,700</td>
<td>85.7 (62.6–96.2)</td>
<td>74.1 (65.0–81.6)</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td><strong>Vaccinated patients (n=128)</strong></td>
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<tr>
<td>IgM</td>
<td>26.3 (10.1–51.4)</td>
<td>99.1 (94.3–100)</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>IgG titer ≥5,400</td>
<td>78.9 (53.9–93.0)</td>
<td>85.3 (77.0–91.1)</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>IgG titer ≥5,000</td>
<td>84.2 (59.5–95.8)</td>
<td>83.5 (74.9–89.7)</td>
<td>p&lt;0.001*</td>
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*Fisher’s two-tailed exact test.
The differential diagnosis achieved by PCR for adenovirus showed no positive results. The RT-PCR for enterovirus or herpes virus was negative in all mumps RT-PCR-positive patients, but positive in seven mumps RT-PCR-negative patients (5.1% of all patients and 6.0% of the group of RT-PCR mumps-negative patients).

**DISCUSSION**

Laboratory diagnosis of mumps can be carried out using classical methods, such as virus isolation in cell culture, or by seroconversion or a four-fold increase in the antibody titer (19). However, low recovery of mumps in cell culture, the need for multiple cell lines (20), as well as difficulties in obtaining paired serum samples to assess seroconversion (21), limit the usefulness of these approaches. RT-PCR constitutes an effective and accepted tool. It has shown itself to be useful in the diagnosis of the most severe cases (16), in the study of outbreaks (1), and provides molecular identification of mumps virus genotypes (22, 23). A drawback of these molecular techniques is that – owing to their requirements and laboriousness – they are usually outside the scope of most clinical laboratories. Under these conditions, laboratory confirmation of mumps infection is frequently attempted by detection of specific IgM (3, 24); this method has been shown to be a good confirmation tool in unvaccinated patients (24). Conversely, it can present a high number of false-negative results in previously immunized subjects.

In the present study the proportion of IgM-positive results in unvaccinated patients was significantly higher than for immunized patients (66.7% versus 4.7%). IgM detection was highly specific (>95%) for diagnosis of mumps infection with independence of vaccination status. In contrast, its sensitivity was very poor, especially in previously vaccinated patients (only a third of the total RT-PCR-positive patients studied, and only a fifth of these vaccinated cases were diagnosed by specific IgM detection).

Cases in vaccinated subjects can be characterized by the presence of higher IgG titers, although not necessarily protective, in the absence of IgM (25). In previously vaccinated patients suffering from parotitis, higher levels of specific and high avidity IgG antibodies can be found, which is characteristic of a secondary immune response (14, 25). In this study, an increasing trend respecting the IgG-antibody logarithmic titers after start of infection among RT-PCR-positive patients (including previously vaccinated) could be observed. This trend was not observed in RT-PCR-negative patients, thus confirming that mumps diagnosis could be made on rising IgG titers.

As shown by the ROC curve analysis, the sensitivity for detection of high IgG titers was significantly greater that the IgM determination. However, not a single IgG cut-off could be universally applied. Detection of elevated titers of IgG, although not sensitive enough to be considered a good diagnostic test, appears to be an evident improvement compared with IgM serologic diagnosis (at least three out of every four patients were diagnosed by IgG titration). Moreover, the application of an IgG cut-off based on elevated titers detected most (>85%) of the mumps cases confirmed by RT-PCR but with IgM-negative results. This fact is important bearing in mind that these patients would not be included as mumps cases if only conventional serologic criteria were adhered to.

In this study, RT-PCR was chosen as the reference on the basis of its high specificity. This method is capable of detecting 0.001 PFU/ml and 0.005 50% tissue culture-infective dose/ml (16). Nevertheless, the moderate specificity obtained in the detection of higher IgG titers could be due in part to the possible inclusion of real mumps cases that were not detected by RT-PCR. All patients showed symptoms that were compatible with parotitis, and some of the RT-PCR-negative patients had IgM-positive results. As expected, a delay in the sampling time (although with no significant statistical difference) in the RT-PCR-negative patients groups was observed. It has been postulated that the mumps viral load decreases after 3 days of infection and is lower in patients serologically diagnosed as having secondary vaccine failure (26).

In some settings the confirmation of suspected mumps cases is only 10% (3). Although other viruses can be the cause of a disease with similar symptoms (3, 27), a wide differential diagnosis in vaccinated populations shows that in more than 85% of cases it is not possible to con-
firm other etiologies (27). In the present study, a positive genomic amplification to other viruses was only obtained in seven patients.

Notifications of mumps can seriously underestimate the real data on the disease (28), and usually only a small proportion of notifications are laboratory confirmed (29). Furthermore, the lack of an IgM response in vaccinated subjects can even mean that the incidence in populations with a high rate of immunization coverage is misjudged. In these situations, IgG titration can be a complementary diagnostic tool to IgM detection. Due to the variability in EIA tests, this strategy may be difficult to implement in practice. The method used here is a well-standardized one, providing good day-to-day performance (30). It is recommended that a convalescent sample be studied to verify IgG seroconversion when no serological conclusive results are obtained in an acute serum. However, as known, it is difficult to obtain paired serum samples; therefore, it was not possible to demonstrate serological changes. In a diagnostic setting, methods that give informative and reproducible results are needed. At the moment the only tools that seem to be absolutely specific are virus isolation and RT-PCR. Nonetheless, these methods are not available in most laboratories. According to our results, the low IgM-antibody sensitivity in persons previously vaccinated suggests that the detection of high IgG titers may be used as a complementary marker of mumps infection in immunized patients. Ideally, RT-PCR should be used together with serology. Under all circumstances further studies are needed to define the role of IgG quantification in the diagnosis of mumps virus infection in previously vaccinated subjects.

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