SHORT COMMUNICATION

IgG AVIDITY IN THE DIAGNOSIS OF ACUTE ROSS RIVER VIRUS INFECTION

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Ross River virus (RRV), an alphavirus, is a major cause of epidemic polyarthritides in Australia and the South Pacific region (Doherty *et al.*, 1971; Mudge and Aaskov, 1983; Cloonan, 1990; Aaskov *et al.*, 1981; Rosen *et al.*, 1981; Tesh *et al.*, 1981). The disease is transmitted by mosquitoes and is mainly characterised by rheumatic manifestations (arthritis and soft tissue rheumatism), rash and constitutional symptoms such as fever, fatigue, and myalgia (Cloonan, 1990).

Laboratory diagnosis of RRV infection is usually made by demonstrating specific IgM antibodies in a single sera and/or a rise in IgG titre in paired sera (Lambkin and Williams, 1984; Oseni *et al.*, 1983; Carter *et al.*, 1985). However, the serological diagnosis of primary infection can be complicated by the lack of appropriately timed sera or the persistence of high IgM titres after acute infection. Tests to assess the avidity of specific IgG have been reported to be of value in diagnosing recent primary infection of other diseases such as rubella (Thomas and Morgan-Capner, 1988), toxoplasma (Holliman *et al.*, 1994), hantavirus (Hedman *et al.*, 1991), and Epstein-Barr virus (de Ory *et al.*, 1993). In order to evaluate IgG avidity as a marker of primary RRV infection, we have used 8M urea as an avidity diluent to investigate IgG reactivity in a commercially available ELISA.

Sera tested represented 116 cases of RRV infection:

- 59 cases of acute infection where seroconversion was demonstrated in paired sera collected 1–17 weeks apart (mean 4 weeks) and an elevated IgM response was shown. The second of the paired sera (IgG and IgM positive) was tested;
- 38 cases of past infection where an IgG but not an IgM response persisted (IgG positive, IgM negative);
- 19 cases of past infection where the IgM response was persistent for 7 months to 8 years after infection (IgG and IgM positive);
- serial samples from 6 of these patients collected for 21–260 days after onset of infection.

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Table 1: Descriptive statistics for different patient groups

Infection	Acute	Past	Past (IgM-)	Past (IgM+)
No. sera	59	57	38	19
mean avidity ^b	19.06	60.46	62.46	56.47
SD ^a	14.76	16.23	18.61	16.32
Avidity $\leq 40\%$ ^c	88%	11%	8%	16%

^a Abbreviations: SD = standard deviation; IgM- = negative IgM response to RRV; IgM+ = elevated IgM response to RRV (persistant IgM);

^b Student t-test: significant difference between mean avidity in patients with acute and past infection (2 sided p-value < 0.0001); no difference between mean avidity in patients with past infection who have elevated or negative IgM (p = 0.2569);

^c Fisher's exact test: significant diffence between proportion of patients with acute and past infection who have an avidity index below 40% (p < 0.0001); no difference between between patients with past infection who have elevated or negative IgM (p = 0.3894).

Figure 1. IgG avidity index in acute and past Ross River virus infection. Diagnostic groups represented are patients with acute infection; patients with past infection who show negative IgM responses; and patients with past infection with persistent elevation of IgM response. Avidity was determined as described in the text.

Figure 2. Changing avidity after onset of Ross River virus infection. Serial data are shown for five patients, each identified by a different symbol.

Serum IgG responses to RRV were determined using commercially available ELISA (PanBio, Brisbane, Australia). Briefly, serum was diluted 1:100 in the diluent provided and 100 μ l was incubated in a RRV antigen coated test strip for 20 minutes at room temperature (RT). After washing with PBS, 100 μ l of anti-human IgG-peroxidase was added to each well for 20 minutes at RT. Strips were washed and developed with TMB substrate before stopping the reaction with 1M H_2SO_4 and reading the absorbance at 450nm in a microtitre plate reader (Absorbance A). In the avidity ELISA, strips were incubated with 100 μ l/well 8M urea (Avidity Reagent, PanBio, Brisbane, Australia) for 5 minutes at room temperature between the serum and conjugate dilutions (Absorbance B). Test strips were washed with PBS before and after the incubation with avidity reagent. The avidity index was calculated using the following formula:

$$\text{Avidity Index (\%)} = \frac{\text{Absorbance B}}{\text{Absorbance A}} \times 100$$

The avidity indices obtained in individual samples are shown in Figure 1, while the descriptive statistics for the different patients groups are summarised in Table 1. There was a significant difference between the mean avidity index obtained in patients with acute or past RRV infection (Student t-test, $p < 0.0001$) (Table 1). Use of an avidity index of 40% led to an excellent differentiation between acute and convalescent infection

(Fishers Exact Test, $p < 0.0001$), with 88% of patients with acute infection (52/59) having low avidity IgG responses (sensitivity 88%), compared to only 11% of patients with past infection (6/57) (specificity 89%). Of those with past infection, 8% with negative IgM responses (3/38) and 16% of those with persistent elevation of IgM (3/19) had low avidity IgG responses, and there was no statistical difference between the avidity in these groups (student t-test on mean, $p = 0.2569$; Fisher's Exact test on proportion, $p = 0.3894$). The profiles of changing avidity for 50–250 days after the onset of RRV infection are shown in Figure 2. The avidity index was low (<40%) in the first sample collected from these patients (between 1 and 40 days after onset) and this subsequently rose in all patients.

These data suggest that the avidity ELISA procedure described here is a useful additional test for distinguishing between acute and past Ross River virus infection.

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