A latex agglutination assay for the detection of anti-streptokinase antibodies

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The need to re-administer thrombolytic therapy is becoming more frequent. Approximately 20% of patients admitted to hospital with acute myocardial infarction (AMI) have had a previous infarct and an increasing proportion will have previously received a thrombolytic agent. Streptokinase (SK), a 47kD protein produced by Group C B hemolytic streptococci, is a widely used thrombolytic agent because of its ability to directly activate plasminogen [1]. Anti-SK antibodies arise either following streptococcal infection or as a result of therapeutic administration of SK. After SK administration the antibody titre, mainly IgG, falls initially then subsequently rises after day 3-4 to reach high values by day 7-10 [1]. It was originally thought that this high value was maintained for only 2 or 3 months, however significant antibody titres have been detected in some patients up to 4 years after administration [2]. Although the clinical significance of these antibodies is not clear, there is evidence that some anti-SK antibodies can neutralise SK activity in vivo, resulting in thrombolytic failure. A need therefore exists for a test to screen AMI patients on admission to the coronary care unit so that those with increased anti-SK antibody levels can then be administered an alternative thrombolytic agent such as t-PA (tissue plasminogen activator). It is important that the test gives results quickly as the thrombolytic agent needs to be administered as early as possible to confer maximum benefit.

We have developed and validated a rapid latex agglutination test for the detection of anti-SK antibodies in serum. Latex particles are covalently coated with a streptokinase. In the test 30µl diluted human serum is mixed with 30µl SK-coated latex on a glass slide, the slide is then gently rotated for a period of 3 minutes and inspected for evidence of agglutination, which if present indicates the presence of anti-SK antibodies in the sample. Simultaneous analysis of a positive and negative control facilitates reading of the slide. The assay has been validated against a microtitre plate assay for anti-streptokinase antibodies. This plate assay was previously shown to correlate well to a highly sensitive functional assay that measures SK neutralisation activity independently of other circulating inhibitory factors in the sample [4].

Forty-one serum samples were obtained from AMI patients before and at various intervals following streptokinase treatment. They were analysed at a 1/100 dilution on the microtitre plate assay and at 1/10 dilution on the latex agglutination assay. There was excellent agreement between the results of both assays (Fig. 1). In addition the samples that gave a positive result on the latex assay had a mean OD of 1.84 on the plate assay (Range 0.69-3.00) which was significantly different from 0.162 the mean obtained for the negative samples (Range 0-0.78).

Figure 1. Scattergram of microtitre plate assay versus latex assay. The line at 0.6 represents the plate assay cut-off.

Figure 2. Results obtained from the latex agglutination assay with serial dilutions of 5 serum samples.

Five of the positive serum samples were assayed at varying dilutions with the latex agglutination assay and a score assigned depending on the degree of agglutination observed (Fig. 2). Although all samples except one were positive at the 1/10 dilution, there was no correlation between degree of agglutination and level of anti-SK antibody present due to a "hook effect" observed at high antibody concentrations. For an estimate of concentration of antibody it is necessary to assay the sample at a range of dilutions.

We conclude that a convenient and rapid bedside test for the detection of anti-streptokinase antibodies in serum has been developed and validated. This test should prove useful for the rapid screening of AMI patients on admission to the Coronary Care Unit.

References: