นิพนธ์ต้นฉบับ

Development of detection kit for anti-platelet antibodies in patients with thrombocytopenia by modified SPRCA

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Abstract

Detection of anti-platelet alloantibodies in serum of patients with thrombocytopenia is important for helps in diagnosis and investigation as well as specific treatment. Since, platelets provide both common and platelet specific antigens which can induce antibody production, the specificity of alloantibodies is needed to be investigated. Solid phase red cell adherence (SPRCA) is a method of choice in laboratory. There are some limited, however, where it needs intact platelet to be immobilized onto microtiter plates as well as the requirement of rabbit anti-platelet antibody where there is now no available. Moreover, to specify anti-HLA or anti-platelet specific antigen, we have to perform the test twice using chloroquin-treated intact platelet. To develop SPRCA to be user friendly and make it more practical in order to verify the specificity of antibody in one-step mode, we produced rabbit polyclonal anti-platelets (Pooled platelet from 5 healthy blood group O donors) and modified it into 2 different modes of specificity, Non-adsorbed rabbit anti-platelet and HLA-adsorbed anti-platelet antibodies, respectively. By this modification, the positive in both or each well could be used to identify the specificity of antibodies in serum. We also optimized the condition of our modified SPRCA. The optimal condition consists of 1) rabbit anti-platelet antibodies (both form coated in parallel), 20 μg/ml/50 μL/well; 2) blocking solution is 5%BSA-PBST; 3) platelet lysate, 2.5 μg/50 μL/well; 4) Rabbit anti-human IgG Fc specific, 1:1000, 50 μL/well; 5) human anti-D IgG sensitized red cells is 0.2% in Alsever's solution, 50 μL/well and 6) to observe agglutination of, we have to spin the reaction plate at 2000 rpm for 2 minutes. Detection of anti-platelet antibodies in 81 patients' serum of various diagnosis found that there were 46 positive. Sixty five specimens were confirmed by PSIFT and it was found that there were 25 true positive and 30 true negative. The 6 samples of one-well positive (P-N pattern) which was expected to be anti-HLA were further on investigated by indirect immunofluorescent test with pooled WBC. It was shown that 4 of them were positive to WBC corresponded to expectation. This SPRCA modified in this study showed promising criteria that it may be used in laboratory since it is practical and specificity of antibody detected can be observed in one-step. However, since the specificity was quite low, more samples should be tested for more reliability. **Bull Chiang Mai Assoc Med Sci 2008; 41: 7-14.**

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