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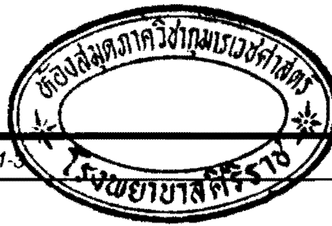

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EDITORIAL

Diagnostic Value of Serological Tests for Melioidosis in an Endemic Area

Melioidosis is a relatively rare infectious disease that is more commonly reported from tropical countries, particularly in the Southeast Asian region.[1,2] However, with the modernization of laboratory facilities, the disease is being diagnosed at an increasingly alarming rate. In fact, it is considered to be a major cause of community-acquired septicemia in northeastern Thailand.[3] The disease is often associated with underlying illnesses or with immunocompromised hosts.[1,4] Melioidosis is caused by *Pseudomonas pseudomallei,* a small bipolar staining gram-negative rod-shaped bacterium. It is non-acid fast, non-sporeforming and motile through the action of polar flagella.[1] Evidence is available suggesting that the organism is a facultative intracellular bacterium that induces acquired cellular immunity in affected individuals.[5,6] However, the bacterium is susceptible to phagocytosis and intracellular killing by immune macrophages.[7] Specific immune serum may facilitate the ingestion and destruction of bacteria by these phagocytic cells.[7]

Currently, isolation and identification of the causative agent from clinical specimens is the only conclusive evidence of melioidosis.[1] However, the bacteriological method is time consuming and may provide results too late for effective therapy. It is apparent therefore that a sensitive, specific, rapid and simple alternative diagnostic method is most urgently needed and that it would be particularly useful in cases with acute septicemia for which there is currently a very high mortality rate. A variety of serological tests for the detection of antibodies to *P.pseudomallei* have been developed for the diagnosis of melioidosis. These include direct bacterial agglutination, indirect hemagglutination (IHA), complement-fixation (CF), immunofluorescence (IFA) and an enzyme-linked immunosorbent assay (ELISA).[8-14] Most of these methods meet the above criteria for a good diagnostic test, but there are a number of problems associated with the interpretation of the results and also occasionally false-positive reactions have been encountered.[15] This necessitates further refinement of these methods.

The IHA was in the past and still is the most commonly employed serological test for the diagnosis of melioidosis because it is highly sensitive and moderately specific. Moreover, it is relatively simple to perform, requires practically no complicated and expensive equipment and gives rapid results.[12-14] Depending on the protocol used, the method is based on the presence of antibody against polysaccharide or lipopolysaccharide of *P.pseudomallei* antigen in the serum of suspected individuals.[8,11] A majority of patients with culture-proven melioidosis are found to be seropositive and the antibody titers may remain elevated after the disease subsides. Persistence of high antibody titers or rising titers may indicate relapse. On the other hand, healthy individuals in the endemic areas of infection often exhibit high background IHA antibody titers, suggesting that subclinical infections are also common.[1] Altogether, the evidence shows that IHA is a useful indicator of previous exposure to *P. pseudomallei* but that it cannot differentiate past from present infection in people residing in endemic areas of infection. It is possible however to raise the cut-off positive level to improve specificity, but this is done at the expense of sensitivity with a risk of false seronegatives, particularly in those culture-prove patients with acute septicemia. It is also not un-

common to find that these patients and those during the early stage of infection exhibit transient seronegative findings.[12-14] On the other hand, a seropositive finding in clinically suspected patients in non endemic areas of infection is highly indicative of melioidosis, particularly in those with a history of travel into endemic areas.

In an attempt to overcome the shortcomings of the IHA test which measures dithiothreitol-sensitive (presumably, IgM) antibody to the carbohydrate component of *P. pseudomallei,*[10,11] assays for specific IgM antibody against protein antigens have been developed. It was thought that the presence of IgM antibody specific for the protein components would more closely parallel the development of clinical melioidosis. Ashdown and his colleagues in Australia were the first to evaluate the significance of IgM antibody by immunofluorescence (IgM-IFA) and ELISA (IgM-ELISA).[10,11] They found the level of specific IgM antibody by these technics to be a much more accurate diagnostic indicator for active melioidosis. Such a conclusion has been confirmed by Kunakorn and his associates in Thailand.[14] Furthermore, the latter group has examined and compared the 2 IgM antibody assays, i.e., indirect ELISA (IgM-ELISA) and IgM antibody capture ELISA (MAC-ELISA) and found both assays to be equally useful and reliable for the diagnosis of clinical melioidosis. It appears therefore that the methods for IgM antibody detection are highly satisfactory with regard to sensitivity and specificity, and that the results more closely reflect the acute stage of infection. Clinical awareness and the combined use of IHA and IgM antibody by either IFA or ELISA are the keys to early diagnosis of acute septicemic melioidosis where accurate and rapid diagnosis is most urgently needed. However, if IHA is the only test available, an IHA titer of $>1:1280$ is indicative of current infection.[12] On the other hand, if the IHA antibody titer is borderline (e.g., 1:80-1:640), then the serum should be sent for IgM antibody measurement by IFA or ELISA. It is difficult to understand why the IHA test which presumably measures the IgM antibody to the polysaccharide component can not differentiate the clinical disease. It should be recalled that the antibody to lipopolysaccharide of other gram-negative bacilli is also IgM.

Although the IgM antibody assays (IFA or ELISA) currently available are highly sensitive and specific, a minor degree of residual nonspecificity can still be encountered, leaving room for further improvement. The latter can be achieved by the use of a more specific antigen rather than the crude antigen currently available. Ismail and colleaques recently developed a sensitive and specific enzyme immunoassay for the detection of antibody to *P. pseudomallei* exotoxin based on the availability of monoconal antibody specific for the 31 KD toxic component.[16] However, whether or not such an antibody is also synthesized in significant quantity in patients with melioidosis remains to be investigated. We have also observed the presence of antibody reactive with a *P. pseudomallei* component with a similar molecular size (unpublished observations). However, it is not known if this component is identical to the 31 KD exotoxin reported previously by our colleagues in Malaysia.[16]

Alternative to a detection of specific IgM antibody, a method for detection of specific antigen(s) is more desirable with regard to a diagnosis of current infection. The shortcomings of antibody detection are the persistence and high background values in endemic areas. The presence of antigen either in the circulation or in body secretions (e.g., urine) would more closely reflect current infection. A competitive ELISA based on the use of monoclonal antibody has been developed for the detection of *P. pseudomallei* exotoxin.[17] The *in vitro* assay developed could detect the presence of exotoxin at a level of 16 ng/ml and was several orders of magnitude more sensitive than the mouse lethality test. Whether or not the exotoxin is also produced in quantity sufficient for its detection in the patient remains to be investigated. More recently, an avidin-biotin enzyme immunoassay based on a rabbit polyclonal antibody to *P. pseudomallei* protein antigen with a sensitivity to 3.9 ng/ml has been described.[18] A similar approach and results have been achieved in my own laboratory using monoclonal antibodies (unpublished observations). However, one must be aware of the fact that antigenic differences among the various *P. pseudomallei* isolates do occur as demonstrated by Lertmemongkolchai and her associates in this issue. It is nevertheless not too farfetched to expect that with the new technologies and specific reagents currently available, a suitable method for antigen detection will be forthcoming in the very near future. With such developments, the fatality rate of acute septicemia caused by *P. pseudomallei* should be drastically reduced.

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