Clinical Features That Affect Indirect-Hemagglutination-Assay Responses to *Burkholderia pseudomallei*\(^7\)

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Melioidosis, a disease endemic to northern Australia and Southeast Asia, is caused by the soil saprophyte *Burkholderia pseudomallei*. The indirect hemagglutination assay (IHA) is the most frequently used serological test to help confirm exposure to the causative organism. However, despite culture-confirmed disease, patients often have a negative IHA result at presentation and occasionally fail to seroconvert in serial testing. We retrospectively examined results for all patients with culture-confirmed melioidosis from our laboratory between January 1996 and August 2008. One hundred forty patients had a recorded IHA titer at presentation, 71 of which were positive at a titer of 1:40 or greater. Fifty-three patients went on to have subsequent IHAs 1 month or more after presentation. The relationships between IHA responses and clinical features were examined. The presence of bacteremia was significantly associated with a negative IHA at presentation. The coexistence of diabetes was associated with the presence of a positive IHA at presentation. In total, 14 patients (26%) demonstrated persistently negative IHA titers upon serial testing. No clinical factors were found to be significantly associated with this phenomenon. Supplementary testing using melioidosis-specific immunoglobulin G by EIA demonstrated different effects, with only Aboriginal or Torres Straits Islander ethnicity being significantly associated with a positive EIA at presentation. Reasons for these findings are examined, and directions for future research are discussed.

Melioidosis, a disease endemic to northern Australia and Southeast Asia, is caused by the gram-negative soil saprophyte *Burkholderia pseudomallei*. It causes significant morbidity and mortality, with a wide spectrum of clinical presentations. Commonly affected organ systems include the lung and genitourinary tract, but patients can also present with bone and joint infection, isolated bacteremic disease, visceral abscess formation, central nervous system involvement, or soft tissue manifestations (21, 23). Presentation can be in the form of either acute fulminant disease with septicemia or a chronic infection. Well-described risk factors for the development of disease include diabetes mellitus, chronic renal failure, chronic lung disease, and excessive alcohol use. Melioidosis appears to be overrepresented in men aged over 45 years and in the indigenous communities of northern Australia (13). It also shows marked seasonal variation, with peak incidences and poor outcomes associated with heavy rainfall and case clustering related to extreme weather events (8, 12). The immune mechanisms that predispose individuals to disease and the nature of immune-mediated responses to infection are not well characterized. Although cell-mediated immunity is thought to be important in the defense against melioidosis (18), it is interesting that human immunodeficiency virus infection is not clearly associated with an increased risk or adverse outcomes in areas where the two diseases are coendemic (10).

The gold standard for the diagnosis of melioidosis is culture from clinical specimens. However, serological tests have an adjunctive role in certain situations, such as screening travelers returning from areas of endemicity with a febrile illness and aiding in diagnoses when the presentation is unusual (e.g., chronic disease) or when specimens for culture may be unavailable (e.g., deep brain abscesses). It may also provide supporting information if melioidosis is suspected but the organism fails to grow. Simple, rapid, and reliable serological tests for melioidosis hold the possibility of identifying cases earlier and thereby improving outcomes, given that culture and identification of *B. pseudomallei* can be delayed.

The indirect hemagglutination assay (IHA) has been the mainstay of serological testing for melioidosis over many years, and the technique has remained largely unchanged since it was first described over 40 years ago (16). Despite variable levels of sensitivity and specificity, it remains the most commonly employed serological test, with titers of 1:40 or greater considered reactive according to the Australian standard (3). High background rates of seropositivity have been described for areas of endemicity and can affect the utility of the test (22). In north Queensland, rates of background IHA seropositivity in the population are relatively high, ranging from 2.5% in asymptomatic blood donors (20) to 5.7% in samples from the district pathology laboratory (3).

The IHA relies on the agglutination of sheep red blood cells in the presence of serum antibodies to polysaccharide and lipopolysaccharide antigens derived from defined strains of *B. pseudomallei*. Several patterns of serological responses, including late seroconversion, persistently reactive and persistently nonreactive test results, and seroreversion, have been described in a previous study that examined serial IHAs over time (7). The range of titers in seropositive specimens is often wide.

Given the relatively poor test characteristics of the IHA,
other methods to aid in the serological diagnosis of melioidosis have been developed. The enzyme immunoassay (EIA) is a commonly employed method. Tests that detect specific immunoglobulins (Ig) directed against *B. pseudomallei*, from both IgG and IgM classes, are available with a range of antigen preparations. Initial work by Ashdown suggested that the detection of specific IgM antibodies by immunofluorescence more closely reflected the presence of active disease (2). Subsequent development of IgG and IgM enzyme-linked immunosorbent assays, and studies of their use in the diagnosis of clinical disease, supported the concept that IgG can be used as a screening method for melioidosis, with IgM being more reflective of disease activity (5). The combination of IgM testing and IHAs has been shown to provide a sensitivity of 100% and a specificity of 95.4% when used in the diagnosis of acute melioidosis in an area of endemicity (19). However, other studies have found detection of IgG more useful in the serodiagnosis of acute disease (9, 14). PCR-based assays have been developed and appear promising, though lacking in sensitivity (15).

This study aimed to describe the patterns of serological responses to culture-confirmed melioidosis observed in Queensland, Australia. Specifically, we were interested in examining the patient factors associated with the failure to mount a detectable antibody response despite culture-confirmed disease. Failure to mount an IHA response could conceptually correlate with demographic factors (such as age, ethnicity, or gender), comorbid conditions (such as diabetes, renal failure, or steroid use), bacteremia, or chronic disease. Indeed, previous work in northern Australia demonstrated that an initially negative IHA result was predicted by the presence of pneumonia or positive blood cultures or by female gender (7). However, the presence of a persistently negative IHA has not been found to correlate with any measured patient characteristics.

**MATERIALS AND METHODS**

Townsville Hospital, a tertiary referral center for tropical north Queensland, serves a population of approximately 300,000. Melioidosis is a relatively common condition in the region, especially during the rainy season. The Townsville Hospital microbiology laboratory also receives isolates from patients admitted and treated in district hospitals. We retrospectively examined the records of all patients with culture-confirmed melioidosis from clinical specimens in our laboratory that were dated from January 1996 to August 2008. Data on clinical presentation, organ involvement, relevant risk factors, and outcomes were analyzed. Ethical approval for this study was obtained from the Townsville Health Service District Ethics Committee.

The IHA was performed as previously described using sheep red cells sensitized with antigens from five selected strains of *B. pseudomallei* (4). Serum samples were heat inactivated and then incubated with nonsensitized sheep red cells to remove nonspecific agglutination. Titers for sample sera in microwell plates were then determined, and antigen-sensitized red cells were added. The presence of antibody was confirmed by red cell agglutination. A titer of ≥1:40 was considered positive. Nonsensitized ovine red cells were used as controls. A supplementary IgG EIA was also undertaken for the majority of patients at admission. The assay was performed as previously described by Ashdown (5). In summary, the EIA was prepared using antigen derived from eight strains of *B. pseudomallei*. After being cultured for 24 h on Trypticase soy agar, a cell suspension underwent heat inactivation and sonication. The filtered extract was then used to coat microtiter plates. Bovine serum was added to prevent nonspecific binding. Sera from samples and controls were added to the plates, which were then washed and incubated with anti-human IgG horseradish peroxidase conjugate. Absorbance was measured and compared to known negative, high-titer positive, and low-titer positive controls. Results were then expressed in EIA units, with a value of ≤25 considered negative, 26 to 50 equivocal, and >50 positive (5). However, for simplicity, equivocal EIA results were excluded from our analysis.

By the use of many descriptive variables, the data were initially divided into two mutually exclusive categories based on whether the patient displayed a particular descriptive characteristic (e.g., the presence of diabetes). The data on the relationship between clinical features and whether the IHA result was positive or negative were placed into two-by-two contingency tables to produce both an odds ratio and a relative risk. Statistical significance of the odds ratio was tested based on the method described by Jewell (17). Significance of the relative risk was assessed as significant at a P value of <0.05 if the 95% confidence limit did not reach 1 (11). A similar analysis was performed with data relating to the results of EIA for the IgG status of patients upon admission. The data on the relationship between clinical features and serial IHA responses were placed into two-by-four contingency tables. As the average expected frequency was greater than six, a χ² analysis was appropriate. Significant outcomes were repeated by using a log-likelihood ratio for contingency tables (G statistic) as a confirmatory test, with an adjustment for the small sample size (17, 25). Although serial EIA were performed for a number of patients, the sample was too small to perform a meaningful statistical analysis of longitudinal IgG responses.

### RESULTS

During the study period, from January 1996 to August 2008, 168 patients were identified with culture-confirmed melioidosis. Of these, 140 had a recorded IHA titer at presentation (defined as within 5 days of positive culture). Organ involvement and disease characteristics were similar to those previously described for tropical Australia (21). The median age at presentation was 53 years, with a range of 6 to 88 years. Five patients (4%) were in the pediatric age group (defined as ≤15 years of age). Of the total number of patients, 81 (57%) were bacteremic at presentation, 14 (10%) went on to experience true disease relapses, and 32 (23%) died. Overall, the lung was the most common organ involved (Table 1).

Of the 140 patients with an IHA result, only 71 (51%) were positive at ≥1:40 on initial testing, reflecting the poor sensitivity of the IHA for acute disease. The relationships between clinical presentation and IHA results are shown in Table 2. There was a significant (P < 0.05) association between diabetes and a positive test for IHA. Patients with diabetes were twice as likely to be IHA positive for *B. pseudomallei* than those without diabetes. There was a significant negative (relative risk, <1) relationship between the relative risk of a positive IHA and the presence of bacteremia.

A proportion of the patients (41%) had repeat IHAs. Several serological patterns in longitudinal IHA responses were

**TABLE 1. Patterns of organ involvement**

| Organ involvement or other parameter | No. of patients (%)* |
|-------------------------------------|----------------------|
| Lung                               | 79 (56)              |
| Gastrointestinal                    | 22 (16)              |
| Skin and soft tissue               | 21 (15)              |
| Central nervous system             | 10 (7)               |
| Bone and joint                     | 13 (9)               |
| No source identified               | 14 (10)              |
| Liver or spleen                     | 6 (4)                |
| Othera                             | 5 (4)                |
| Bacteremia                         | 81 (57)              |
| Death                              | 32 (23)              |

* Several patients presented with more than one organ system involved; hence, the total number exceeds the patient population of 140, and the percentages do not add up to 100.

* Includes aneurysm tissue, peritoneal fluid, and lymph nodes.
of these, 8 (15%) had seroconversion as seen in an initial negative test, 22 (42%) had persistently positive test results, 14 (26%) had persistently negative test results, and 9 (17%) experienced seroreversion, going from an initially positive result to a negative result upon convalescent testing. Repeat IHAs were performed after a median duration of 8 months (range, 1 to 55 months). The relationships between serial IHA responses and patient factors are shown in Table 3.

There was a significant relationship ($P$ value of $<0.001$ for both the $\chi^2$ test and the G statistic) between serial IHA responses and

| Variable                      | Total no. of patients | No. of patients with indicated IHA result | OR | OR $P$ value | RR | RR 95% CI |
|-------------------------------|-----------------------|------------------------------------------|----|--------------|----|-----------|
|                               |                       | Positive | Negative |    |              |    |           |
| All patients                  | 140                   | 71       | 69       |    |              |    |           |
| Age (yr)                      |                       |          |          |    |              |    |           |
| $\leq 45$                     | 48                    | 24       | 24       | 1.15 | $>0.05$     | 0.98 | 0.68–1.36 |
| $\geq 46$                     | 92                    | 47       | 45       |    |              |    |           |
| Sex                           |                       |          |          |    |              |    |           |
| Male                          | 93                    | 47       | 46       | 0.9 | $>0.05$     | 0.95 | 0.70–1.35 |
| Female                        | 47                    | 25       | 22       |    |              |    |           |
| Ethnicity                     |                       |          |          |    |              |    |           |
| ATSI                          | 54                    | 33       | 21       | 1.89 | $>0.05$     | 1.35 | 0.98–1.81 |
| Non-ATSI                      | 86                    | 39       | 47       |    |              |    |           |
| Diabetes                      |                       |          |          |    |              |    |           |
| Present                       | 50                    | 31       | 19       | 2.04 | $<0.05$     | 1.4  | 1.01–1.86 |
| Absent                        | 90                    | 40       | 50       |    |              |    |           |
| Alcohol abuse                 |                       |          |          |    |              |    |           |
| Present                       | 39                    | 20       | 19       | 1.03 | $>0.05$     | 1.02 | 0.69–1.4  |
| Absent                        | 101                   | 51       | 50       |    |              |    |           |
| Corticosteroid use            |                       |          |          |    |              |    |           |
| Present                       | 13                    | 6        | 7        | 0.9  | $>0.05$     | 0.82 | 0.46–1.44 |
| Absent                        | 127                   | 65       | 62       |    |              |    |           |
| Chronic renal failure         |                       |          |          |    |              |    |           |
| Present                       | 11                    | 3        | 8        | 0.34 | $>0.05$     | 0.52 | 0.18–1.10 |
| Absent                        | 129                   | 68       | 61       |    |              |    |           |
| Immunosuppressive drug use    |                       |          |          |    |              |    |           |
| Present                       | 9                     | 3        | 6        | 0.46 | $>0.05$     | 0.64 | 0.23–1.28 |
| Absent                        | 131                   | 68       | 63       |    |              |    |           |
| Hematological malignancy      |                       |          |          |    |              |    |           |
| Present                       | 4                     | 2        | 2        | 0.97 | $>0.05$     | 0.99 | 0.29–1.71 |
| Absent                        | 136                   | 69       | 67       |    |              |    |           |
| Solid-organ malignancy        |                       |          |          |    |              |    |           |
| Present                       | 5                     | 2        | 3        | 0.64 | $>0.05$     | 0.78 | 0.23–1.54 |
| Absent                        | 135                   | 69       | 66       |    |              |    |           |
| Chronic lung disease          |                       |          |          |    |              |    |           |
| Present                       | 14                    | 7        | 7        | 0.91 | $>0.05$     | 0.95 | 0.50–1.45 |
| Absent                        | 126                   | 66       | 60       |    |              |    |           |
| Immunosuppressive disorders   |                       |          |          |    |              |    |           |
| Present                       | 4                     | 1        | 3        | 0.31 | $>0.05$     | 0.49 | 0.09–1.39 |
| Absent                        | 136                   | 70       | 66       |    |              |    |           |
| Lung involvement              |                       |          |          |    |              |    |           |
| Present                       | 79                    | 38       | 41       | 0.74 | $>0.05$     | 0.86 | 0.63–1.19 |
| Absent                        | 61                    | 34       | 27       |    |              |    |           |
| Bacteremia                    |                       |          |          |    |              |    |           |
| Present                       | 81                    | 30       | 51       | 0.26 | $>0.05$     | 0.53 | 0.40–0.73 |
| Absent                        | 59                    | 41       | 18       |    |              |    |           |

* OR, odds ratio; RR, relative risk; 95% CI, 95% confidence interval.

seen; of these, 8 (15%) had seroconversion as seen in an initial negative test, 22 (42%) had persistently positive test results, 14 (26%) had persistently negative test results, and 9 (17%) experienced seroreversion, going from an initially positive result to a negative result upon convalescent testing. Repeat IHAs were performed after a median duration of 8 months (range, 1 to 55 months). The relationships between serial IHA responses and patient factors are shown in Table 3.

There was a significant relationship ($P$ value of $<0.001$ for both the $\chi^2$ test and the G statistic) between serial IHA responses and
the presence of bacteremia. This result was strongly driven by the facts that the majority (88%) of seroconverted patients were bacteremic and no bacteremic patients seroreverted.

A total of 121 patients (86% of those with an IHA) had supplementary melioidosis-specific IgG testing by EIA on admission. Ethnicity appeared to affect the probability of seropositivity. Those of Aboriginal or Torres Straits Islander (ATSI) origin were more than twice as likely to be IgG positive. The results are summarized in Table 4. In contrast to the IHA results, the presence of diabetes did not seem to affect the chances of a positive EIA result, and bacteremia was not associated with the failure to detect IgG. Of the 14 patients with
persistently IHA-negative results, 5 had detectable IgG by EIA on admission and a further 5 had serial EIA measurements. Of the five with serial EIA measurements, only one had persistently negative serology on both IHA and EIA, two were persistently EIA positive, and two later seroconverted.

**DISCUSSION**

This study reconfirms the poor sensitivity of the IHA in the diagnosis of melioidosis; only 51% of patients with definite disease were seropositive at presentation. Overall, few clinical

| Variable                        | Total no. of patients | No. of patients with indicated IgG result | OR     | OR P value | RR   | RR 95% CI |
|---------------------------------|-----------------------|------------------------------------------|--------|------------|------|-----------|
|                                 |                       | Positive | Negative |          |      |           |
| All patients                    | 121                   | 86       | 35       |          |      |           |
| Age                             |                       |          |          |          |      |           |
| Age ≤ 45                        | 48                    | 24       | 24       | 1.15    | >0.05| 0.98      | 0.68–1.36 |
| Age ≥ 46                        | 92                    | 47       | 45       |          |      |           |
| Sex                             |                       |          |          |          |      |           |
| Male                            | 83                    | 58       | 25       | 0.83    | >0.05| 0.95      | 0.78–1.2  |
| Female                          | 38                    | 28       | 10       |          |      |           |
| Ethnicity                       |                       |          |          |          |      |           |
| ATSI                            | 50                    | 41       | 9        | 2.63    | <0.05| 1.29      | 1.03–1.5  |
| Non-ATSI                        | 71                    | 45       | 26       |          |      |           |
| Diabetes                        |                       |          |          |          |      |           |
| Present                         | 46                    | 36       | 10       | 1.8     | >0.05| 1.17      | 0.93–1.4  |
| Absent                          | 75                    | 50       | 25       |          |      |           |
| Alcohol abuse                   |                       |          |          |          |      |           |
| Present                         | 37                    | 25       | 12       | 0.79    | >0.05| 0.93      | 0.7–1.17  |
| Absent                          | 84                    | 61       | 23       |          |      |           |
| Corticosteroid use              |                       |          |          |          |      |           |
| Present                         | 8                     | 5        | 3        | 0.66    | >0.05| 0.87      | 0.43–1.23 |
| Absent                          | 113                   | 81       | 32       |          |      |           |
| Chronic renal failure           |                       |          |          |          |      |           |
| Present                         | 5                     | 5        | 0        | NA      | >0.05| 1.43      | 0.81–1.4  |
| Absent                          | 116                   | 81       | 35       |          |      |           |
| Immunosuppressive drug use      |                       |          |          |          |      |           |
| Present                         | 6                     | 4        | 2        | 0.8     | >0.05| 0.93      | 0.42–1.29 |
| Absent                          | 115                   | 82       | 33       |          |      |           |
| Hematological malignancy        |                       |          |          |          |      |           |
| Present                         | 4                     | 3        | 1        | 1.23    | >0.05| 1.06      | 0.42–1.36 |
| Absent                          | 117                   | 83       | 34       |          |      |           |
| Solid-organ malignancy          |                       |          |          |          |      |           |
| Present                         | 5                     | 3        | 2        | 0.6     | >0.05| 0.84      | 0.32–1.25 |
| Absent                          | 116                   | 83       | 33       |          |      |           |
| Chronic lung disease            |                       |          |          |          |      |           |
| Present                         | 11                    | 5        | 6        | 0.3     | >0.05| 0.62      | 0.29–0.999|
| Absent                          | 110                   | 81       | 29       |          |      |           |
| Immunosuppressive disorders     |                       |          |          |          |      |           |
| Present                         | 1                     | 1        | 0        | NA      | >0.05| 1.29      | 0.27–1.3  |
| Absent                          | 120                   | 120      | 35       |          |      |           |
| Lung involvement                |                       |          |          |          |      |           |
| Present                         | 69                    | 46       | 23       | 0.6     | >0.05| 0.87      | 0.71–1.09 |
| Absent                          | 52                    | 40       | 12       |          |      |           |
| Bacteremia                      |                       |          |          |          |      |           |
| Present                         | 70                    | 46       | 24       | 0.53    | >0.05| 0.84      | 0.69–1.05 |
| Absent                          | 51                    | 40       | 11       |          |      |           |

*OR, odds ratio; RR, relative risk; 95% CI, 95% confidence interval.*
studies have described longitudinal patterns of serological responses in patients with culture-confirmed disease. We aimed to determine which patient characteristics predisposed them to fail to mount a detectable antibody response or predicted subsequent changes in IHA titers over time. Previous work from Darwin, Australian Northern Territories, found that female sex, pneumonia, and chronic renal disease correlated with an initially negative IHA (7); these findings were not replicated in our study. However, a robust finding, from both our study and the work from Darwin, appears to be the presence of bacteremia predicting an initially negative IHA. The rapid progression of disease and the high bacterial load in patients with bacteremia may have contributed to an inadequate time for development of an antibody response at the time of presentation. Bacteremia was the only significant factor affecting serial IHA patterns. Most patients who later seroconverted were bacteremic at presentation and no bacteremic patients seroreverted, indicating that although the rapid onset of disease may provide little time for detectable seroconversion, the stimulus for subsequent antibody production is significant and long lasting.

The finding that diabetes predicts a positive IHA at presentation is interesting. Given the known effects of diabetes on cell-mediated and humoral immunity, a correlation with a negative IHA would perhaps be expected. However, it is plausible that the immunosuppressive effects of diabetes may predispose patients to infection with less-virulent strains of B. pseudomallei; thus, less-fulminant infection may allow time for antibody production and seroconversion as detected by IHA. The differences between our findings and those from Darwin, where gender and lung involvement were independent predictors of a negative IHA, may reflect variable patient epidemiology, differences in assay characteristics (including antigen preparation), or differences in sample sizes.

The majority of our patients had a supplementary EIA upon admission. The EIA showed improved sensitivity (71%) compared to that of the IHA (51%). These test characteristics are similar to previous findings for the IHA (7, 24) but display marginally inferior sensitivity for the EIA compared to that in previously published data (5, 6). ATSI status was found to be significantly associated with a positive IgG at presentation. It should be noted that the odds on ethnicity and a positive IHA was high (1.89) for ATSI patients but failed to reach statistically significant levels. A tendency for ATSI patients toward seropositivity may result from delayed presentation, allowing more time for the development of detectable antibodies.

The effects of bacteremia and diabetes on antibody responses tested by IHA were not replicated in EIA. The discordance between the tests in the presence of bacteremia may result from the improved sensitivity of the EIA platform to detect low-level or different antibodies than those detected by the IHA. The difference in the context of diabetes is harder to explain but possibly reflects the different antigens used in the two assays. The IHA uses a poorly characterized polysaccharide antigen derived from heat-killed whole bacteria, whereas the EIA employs a sonicated aqueous protein extract (5). Host-pathogen interactions in melioidosis are altered in the presence of diabetes. Clarifying the nature of these interactions and identifying antigens that are expressed early in clinical disease would be of potential benefit both to our understanding of the pathogenesis of melioidosis and to serodiagnosis. Recent work has attempted to develop well-defined recombinant antigens, such as BipD, BPSL0972, or OmpA, for use in an EIA format. Of these, the use of OmpA shows encouraging test characteristics, with sensitivity and specificity of 95% and 98%, respectively (1).

The presence of persistently negative IHA results despite culture-confirmed disease raises a question as to why such patients fail to mount a detectable antibody response. Although no specific factors that predicted this outcome could be identified in our study, it is possible that antibodies are being elaborated but not against the antigens present within the limited strains employed in the IHA and thus are not detected. Alternatively, the antigens contained and presented within the IHA format may not be suitable for the detection of all B. pseudomallei-specific antibodies in test sera. Thus, the lack of detectable antibody represents a failure of the test and not a failure of humoral immunity. That many of these seronegative patients have or develop IgG detectable by EIA supports this view. We intend to further examine this phenomenon by including the cultured isolates from patients that show persistently negative IHA titers in a new corresponding antigen preparation in both IHA and EIA formats. Positive tests in the presence of antigens from their own cultured isolate would confirm that the IHA employs a too-limited array of antigen, leading to falsely negative results.

Due to the retrospective nature of this study, there is some lack of uniformity in the data. For instance, timings of repeat serological testing were variable. True patterns of serial IHA responses may therefore be inaccurately represented. Some clinical features, such as chronic disease presentations or the presence of septic shock, were not recorded in the data set and may have provided useful information. Although this series of patient data was the second largest collected in Australia in the study of melioidosis, the overall numbers are still relatively small, especially when statistical analyses involve comparisons between even smaller subgroups. Thus, weak effects are hard to detect.

In summary, this study reiterates the poor sensitivity of the IHA in the context of culture-confirmed melioidosis. The presence of diabetes appears to significantly predict the presence of a positive IHA at presentation. The presence of bacteremia significantly predicts a negative IHA, with subsequent seroconversion the most likely outcome. However, these effects were not replicated when tested against melioidosis-specific IgG by EIA.Persistently negative serology by IHA is not associated with any measured patient characteristic and may reflect the lack of corresponding antigens contained within the IHA preparation.

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