Usefulness of staphylococcal serology

The successful treatment of systemic infection with *Staphylococcus aureus*, such as septicemia and endocarditis, depends very much on confirming the diagnosis by culture and antibiotic sensitivity testing to guide the choice of antimicrobial agent, but is also influenced by whether infection is complicated, i.e., the development of metastatic infection, or not. The absence of a positive culture represents a considerable clinical dilemma, as confirmation of the correct choice of agents is absent and the duration of therapy remains a matter of clinical judgement; patients with complicated infection require longer courses of treatment. Therefore, there has been much interest over the last two decades in developing staphylococcal serology to confirm a diagnosis in culture-negative patients, and to help distinguish uncomplicated from complicated infection [1]. In 1987 the American Society for Microbiology published techniques for the detection of antibodies to teichoic acid, and recommended that sera be taken at the time of the first isolation of the organism and 14 days later when diagnosing *S. aureus* endocarditis [2]. In a carefully conducted study of staphylococcal serology, Colque-Navarro and colleagues conclude by predicting that staphylococcal serology will be used to monitor treatment, detect relapse or re-infection, and identify patients at high risk of a complicated course [3]. However, this optimistic view does not take account of certain problems with the routine use of serology to guide management.

The authors collected sera on the day of admission, 3 days later and once a week thereafter from 63 patients with *S. aureus* septicemia, and antibodies against α-toxin, teichoic acid and lipase were measured. In all, 40% and 60% of patients had high levels of antibodies against α-toxin and teichoic acid, respectively, but 25% of patients did not produce antibodies to any of the antigens within 30 days of diagnosis [3]. Furthermore, patients with low initial antibody levels and patients with complicated infection usually had a weaker, not stronger antibody response – although complicated bacteraemia was also sometimes characterised by a constantly high titre. Their study found that 88% of patients with uncomplicated bacteraemia had positive results overall, but only 68% of those with complicated infection were positive, the very group of patients for whom guided antibiotic treatment is needed. The response to lipase was somewhat disappointing (only 49% positive between 8 and 14 days after onset of infection), in contrast to a previous study from this group, when >60% of patients were positive for antibodies to lipase [4].

The humoral response to *S. aureus* infection is complex and despite the fact that α-toxin, teichoic acid and lipase are widely recognised as staphylococcal virulence determinants, the production of antibodies to one or more antigens is variable. Indeed, Colque-Navarro and colleagues acknowledge that the kinetics of the antibody response differs greatly between patients. Previous staphylococcal infection or non-specific antibody responses may be significant factors in partly explaining the high initial antibody levels. In contrast, failure to mount an antibody response may be caused by the impaired immunity of advancing age, poor expression of virulence determinants by the infecting strain, or changes in the immune response resulting from the infection itself. In a study of six patients with *S. aureus* endocarditis, antibodies to *S. aureus* and *Escherichia coli* heat-shock proteins were detected by Western immunoblots, but a similar response was observed in sera from patients with other forms of endocarditis [5]. Whilst heat-shock proteins may not be as specific as the staphylococcal antigens studied by Colque-Navarro and colleagues, these results emphasise that the serological response to staphylococcal infection is complex, partly because individuals are exposed to *S. aureus* from birth.

This well-conducted prospective study involving clear definitions of infection, the collection of multiple sera from infected patients and the use of reliable assays against three antigens performed in duplicate, still casts doubts over the routine use of staphylococcal serology in the diagnostic laboratory as a confirmatory or complementary investigation to culture, despite a clear clinical need for such an alternative.

**References**

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Dr P. COLQUE-NAVARRO & Dr R. MÖLLBY replies

Dr Hilary Humphreys’ analysis of some of the data presented in our study is correct, and we appreciate the interest in this area. Two things seem to trouble Dr Humphreys: (1) how can the authors claim in the discussion that there would be a place for these tests in the routine diagnostic laboratory as a confirmatory or complementary investigation and (2) why are the number of patients yielding positive results with lipase as antigen lower than in an earlier report from the same laboratory?

The first point raises many interesting questions about diagnostic laboratory work, e.g., what are the criteria for a routine diagnostic test to be considered useful? Firstly, the test reagents must be available and the test must be reasonably priced and reproducible. Secondly, there must be a diagnostic value in it. But how do we evaluate that quantitatively? Sensitivity and specificity? Yes, but what are the limits, and how does it help the clinician? Positive and negative predictivity are of course of interest, but they require a precise knowledge of the sample material to be tested, including the true prevalence, and the exact results of the negative cases in this particular patient material.

It has been suggested that ‘likelihood ratio’ (LR) would be helpful [1]. The positive LR would indicate the ratio between the probability of a test being positive (T+) when the patient has the disease in question (D+) and the probability of a test being positive when the patient does not have the disease (D-). The corresponding negative LR would give the corresponding ratio for a negative test result, and thus these ratios could be expressed as:

\[
LR^+ = \frac{p(T+/D+)}{p(T+/D-)}
\]
\[
LR^- = \frac{p(T-\neg/D+)}{p(T-\neg/D-)}
\]

These ratios are not dependent upon the prevalence figure, and they will be infinite when a ‘wrong’ test answer does not occur (division by zero). In this case the test would be ‘absolute’ or pathognomonic, but unfortunately such tests are seldom available or are subject to other limitations. In fact, since absolute (pathognomonic) criteria are seldom available, most clinical diagnosis may be looked upon as a summary of several LRs obtained from oral, physical and laboratory examinations. When this ‘sum’ is considered to be high enough, the diagnosis is given to the patient.

In bacterial serological diagnosis the value of a single serum sample has to be carefully examined [2]. It is always preferable that at least two adequately spaced sera are taken to detect a possible rise in antibody titres. Such a rise might be considered pathognomonic per se, but unspecific antigen stimulation may occur. However, in most cases only a single serum sample is available and as Dr Humphreys points out, practically all individuals have antibodies because of earlier exposure [2–5]. Here, careful studies on age-correlated ‘cut-off’ limits are necessary, including studies on healthy controls, control patients with similar disease and true patients [6].

Although the article referred to was not concentrating on serological diagnostic aspects, some diagnostic values may be calculated for the use of α-toxin or teichoic acid, or both, as antigens in a routine test (lipase did not add anything in this study). For this purpose we studied patients with septicaemia of non- *S. aureus* origin together with those with fever without septicaemia (n = 44) as the negative patient group for comparison (Table 1).

From such a table it can be concluded that:

- If the test is performed on two or more sera and a high titre or a rise in titre, or both, is considered positive, a positive test result would increase the probability of *S. aureus* septicaemia in the patient 3.3 times and a negative test-result would decrease it 3 times.
- If a rise in titre can be verified, this result is pathognomonic, i.e., it does not occur in the control group (LR+ = ∞).
- If only single sera are available, the positive LR goes from 2.6 to 4.7 with time.

These conclusions and figures contain all the problems encountered with the sometimes limited specificity and

### Table 1. Diagnostic interpretation of serum titres

<table>
<thead>
<tr>
<th>Days after onset of disease</th>
<th>Sepsis patients (n)</th>
<th>Control patients (n)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive likelihood ratio (LR+)</th>
<th>Negative likelihood ratio (LR-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High titre</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–7</td>
<td>50</td>
<td>44</td>
<td>58</td>
<td>77</td>
<td>2.6</td>
<td>1.8</td>
</tr>
<tr>
<td>8–14</td>
<td>47</td>
<td>26</td>
<td>75</td>
<td>77</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>15–30</td>
<td>37</td>
<td>13</td>
<td>73</td>
<td>85</td>
<td>4.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Rise of titre</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14</td>
<td>39</td>
<td>26</td>
<td>46</td>
<td>100</td>
<td>∞</td>
<td>1.9</td>
</tr>
<tr>
<td>0–30</td>
<td>33</td>
<td>13</td>
<td>52</td>
<td>100</td>
<td>∞</td>
<td>2.1</td>
</tr>
<tr>
<td>High and/or rise during day 0–30 (any serum)</td>
<td>47</td>
<td>44</td>
<td>75</td>
<td>77</td>
<td>3.3</td>
<td>3.0</td>
</tr>
</tbody>
</table>
sensitivity under different sampling and analysis conditions. Whether these results may be considered a valuable contribution to the diagnostic arsenal for this disease is a matter of opinion. However, the information obtained from staphylococcal serology has been deemed valuable in Sweden, where all major clinical microbiological laboratories have used this test in their routine serology for >12 years.

The use of the test for monitoring treatment and identifying patients at high risk as suggested by the authors, has to be validated through further studies. However, Ericsson et al. used the test for direct decisions regarding treatment in staphylococcal pneumonia in cystic fibrosis patients, while the test may also have a role in confirmatory diagnosis [7].

The difference in serological response to staphylococcal lipase in this study (49%) as compared with a previous study by Tyski et al. (60%) [8] may be caused by natural variations, but is most likely due to the fact that the earlier study used retrospective (and selected) patient samples, while the samples in the present study were collected consecutively.

In contrast to the positive findings published elsewhere about the influence of wound licking by dogs [3], we report a case, in which wound licking might have been a co-factor in the progression of wound infection leading to the amputation of a toe in a diabetic patient.

In October 1996, a 48-year-old man suffering from long-term non-insulin-dependent diabetes mellitus was admitted with erysipelas on both lower legs and with ulcers on both feet. Fasting blood glucose level and a co-factor in the progression of wound infection report a case, in which wound licking might have been postulate that several growth factors derived from the bacterial colonisation with taken from the ulcers showed, in addition to the usual growth of Prevotella bivia, P. oralis, P. loescheii, Trichosporon beigelii and Candida guilliermondii which are present in the oral flora of animals and have been isolated from infected animal bites [4, 5]. The patient had allowed his dog to lick the ulcers regularly. Despite intravenous antimicrobial therapy (pipercillin 4.0 g three times daily for 2 weeks, ciprofloxacin 200 mg twice daily and clinda-

Foot infection with *Prevotella bivia*, *P. oralis* and *P. loescheii* after wound licking

Recently, there have been some reports about the possible beneficial effect of wound licking for the promotion of wound healing by antimicrobial substances like nitric oxide [1]. Playford and co-workers postulate that several growth factors derived from the saliva of animals may further contribute to wound closure [2].

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In October 1996, a 48-year-old man suffering from long-term non-insulin-dependent diabetes mellitus was admitted with erysipelas on both lower legs and with ulcers on both feet. Fasting blood glucose level and glycosylated haemoglobin were elevated (171 mg% and 9.2%, respectively) and radiography revealed osteomyelitis of the metatarsalia on the right foot. Specimens for bacteriological and fungal cultures taken from the ulcers showed, in addition to the usual bacterial colonisation with *Staphylococcus aureus* and streptococci, growth of *Prevotella bivia, P. oralis, P. loescheii, Trichosporon beigelii* and *Candida guilliermondii* which are present in the oral flora of animals and have been isolated from infected animal bites [4, 5]. The patient had allowed his dog to lick the ulcers regularly. Despite intravenous antimicrobial therapy (pipercillin 4.0 g three times daily for 2 weeks, ciprofloxacin 200 mg twice daily and clinda-

mycin 1200 mg twice daily for 3 weeks), antifungal treatment (terbinafin 250 mg once daily by mouth and topical ketoconazole cream), rheological medication (aldroadestadil 40 μg twice daily) and topical anti-septics, amputation of the fifth toe became necessary. On the eighth postoperative day, the patient was discharged in an acceptable clinical condition and no further ulcers have occurred.

Although we cannot rule out that the amputation in this case would have become necessary because of the poor metabolic condition of the patient, we do not recommend wound licking by animals for patients with reduced microcirculation.

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