RAPID SERODIAGNOSIS OF *STAPHYLOCOCCUS AUREUS* SURGICAL SITE INFECTION FOLLOWING MEDIAN STERNOTOMY.

RUNNING TITLE: SEROLOGICAL DIAGNOSIS OF STERNAL WOUND INFECTIONS

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Summary

Objectives: To determine the sensitivity and specificity of a novel ELISA for the serodiagnosis of surgical site infection (SSI) due to staphylococci following median sternotomy.

Methods: Twelve patients with a superficial sternal SSI and 19 with a deep sternal SSI due to S. aureus were compared with 37 control patients who also underwent median sternotomy for cardiac surgery but exhibited no microbiological or clinical symptoms of infection. A further 5 patients with sternal SSI due to coagulase-negative (CoNS) staphylococci were studied. An ELISA incorporating a recently recognised exocellular short chain form of lipoteichoic acid (lipid S) recovered from CoNS, was used to determine serum levels of anti-lipid S IgG in all patient groups.

Results: Serum anti-lipid S IgG titres of patients with sternal SSI due to S. aureus were significantly higher than the control patients ($P<0.0001$). In addition, patients with deep sternal SSI had significantly higher serum anti-lipid S IgG titres than patients with superficial sternal SSI ($P=0.03$). Serum anti-lipid S IgG titres of patients with sternal SSI due to CoNS were significantly higher than the control patients ($P=0.001$).

Conclusion: The lipid S ELISA may facilitate the diagnosis of sternal SSI due to S. aureus and could also be of value with infection due to CoNS.

Key words: Sternotomy, surgical site infection, S. aureus, ELISA.

Introduction

Surgical site infection (SSI) following median sternotomy is an uncommon but potentially fatal complication of cardiac surgery. The reported incidence of sternal SSI varies widely and ranges between 0.9% and 20%.[1] Furthermore, the attributable costs of sternal SSI following median sternotomy are particularly high. Indeed, patients with a deep chest wall SSI following coronary artery bypass grafting (CABG) have a 20 day increase in hospitalisation, attributable costs of 20,000 US dollars and a mortality rate of 22%.[2-3]

Early and accurate diagnosis of sternal SSIs is crucial for successful treatment. Grossi and colleagues (1985) suggested that 80% of sternal SSI may be eradicated by simple surgical debridement or closed antibiotic irrigation if the diagnosis is established within 20 days. However if diagnosis is delayed, surgical debridement followed by muscle flap reconstruction may be required.[4] Sternal SSI is often diagnosed by the presence of symptoms of local inflammation including; purulent exudate, erythema, tenderness, pyrexia, elevated levels of serum inflammatory markers [5] and by an abnormal computerised tomography scan.[6] Diagnosis of sternal wound infections is difficult in the early postoperative period due to the general inflammatory response that follows extracorporeal circulation.[7] Use of inflammatory markers to aid diagnosis during this period is therefore of limited value.[8,9] Most imaging techniques which are used for the diagnosis of sternal SSI are based on the visualisation of anatomical structures and are limited in their diagnostic value as infection is indistinguishable from surgical artefacts and the presence of oedema, haematoma or haemorrhage which are often present in normal patients recovering from cardiac surgery.[10]

The microorganisms most commonly recovered from sternal SSI are S. aureus and coagulase-negative staphylococci (CoNS) which account for 32% and 23% of sternal SSI respectively.[11] Microbiological culture of clinical material from the sternal SSI is often required to confirm the clinical diagnosis. However this may be complicated by the use of prophylactic antibiotics used for cardiac surgery. In addition, interpretation of sternal cultures yielding CoNS, a major cause of sternal SSI, is complicated as the isolated microorganisms may represent skin contamination.

Serological diagnosis of sternal SSI due to S. aureus relies mainly on the widely used anti-staphylolysin test (ASTA). However, the assay lacks sensitivity and is only designed for the diagnosis of infection due to S. aureus and not CoNS.[12] Enzyme-linked immunosorbent assay (ELISA) is a frequently utilised, inexpensive, simple serological technique for the serodiagnosis of several types of infections. We have recently developed a rapid, indirect ELISA incorporating lipid S, a novel exocellular short chain form of lipoteichoic acid probably produced by most strains of staphylococci, for the diagnosis of deep-seated staphylococcal infection.[13-15] The ELISA has previously been used to facilitate the diagnosis of central venous catheter related bloodstream infection,[13,14] endocarditis,[16] prosthetic joint infection[17] and pyogenic spondylodiscitis.[18]

In this current study, serum antibody levels of anti-lipid S IgG were determined in cardiac surgery patients with clinical and microbiological evidence of S. aureus and CoNS sternal SSI following median sternotomy and compared to patients with no infectious complications following the same type of surgical procedure.

Materials and Methods

Patients

Thirty-one patients at the Cardiac Surgery Critical Care Unit, University Hospital, Birmingham, UK who were diagnosed as having a S. aureus sternal SSI following elective cardiac surgery were entered into the study. A further 5 patients with sternal SSI due to coagulase-negative (CoNS) staphylococci were included. Patients with other foci of infection were excluded from the study. CDC guidelines were utilised to determine sternal SSI.[19] These patients were compared with 37 randomly selected cardiac surgery patients with no evidence of infectious complications following surgery. In both patient groups, only patients with no evidence of infection in the 6 months prior to surgery were recruited. Clotted blood samples were obtained five days post-cardiac surgery from control patients (immediately prior to discharge in patients with no post-operative complications) and from infected patients at the time sternal SSI was diagnosed microbiologically. Serum IgG titres to lipid S was subsequently determined on these samples. Ethical committee approval was obtained prior to undertaking the study.

Preparation of lipid S-coated plates

The lipid S-coated plates were prepared as outlined previously.[13-15] In brief, the lipid S antigen was prepared from seven pooled strains of CoNS, all isolated from patients with proven staphylococcal infection. Antigen was recovered by gel permeation chromatography (Superose 12) from broth culture. Purified antigen, in carbonate buffer, was used to coat the microtitre plate.

Lipid S ELISA

The lipid S ELISA was performed as outlined by Worthington and colleagues.[13] Patients' sera were diluted 1 in 6400 in TBS/Tween buffer and 100μl was added to each well of the plate. After incubation for two hours at 37°C, excess serum was removed and the plates were washed with TBS/Tween buffer. Antihuman IgG conjugate (Sigma, Poole, Dorset, UK); diluted 1 in 5000 in TBS/Tween buffer was then added to each well and incubated for one hour at 37°C for the detection of bound IgG. After removal of the conjugate, 100μl of chromogenic substrate was added to each well. Following 25 minutes of incubation at 37°C, the reaction was stopped by the addition of 100μl sulphuric acid (1M) after which, the optical density was read at 450nm. All sera, including positive and negative controls were tested in duplicate.

Statistics

Titres were compared using the Mann-Whitney U and Spearman Rank tests.

Results

Patients

Sevently-three patients were recruited into the study and were classified as follows; 14 with superficial sternal SSI (12 due to S. aureus and 2 due to CoNS), 22 patients with deep sternal SSI (19 due to S. aureus and 3 due to CoNS) and 37 control patients with no microbiological or clinical evidence of infection. Patients first presented with superficial sternal SSI a mean of 15 days post-operatively (range 4-30) whereas patients with deep sternal SSI were diagnosed on average, 29 days post-operatively (range 2-270) Patient demographics are given in table I.

Serology

Anti-lipid S IgG titres in the patient groups are shown in table II. A scatter graph demonstrating the range of serum IgG titres to lipid S is given in figure 1. The serum IgG titres of patients with S. aureus sternal SSI were significantly higher than the control group ($P<0.0001$). In addition, patients with deep sternal SSI had significantly higher serum anti-lipid S IgG titres than patients with superficial sternal SSI ($P=0.02$). All five patients with sternal SSI due to CoNS had positive anti-lipid S IgG titres. Indeed, serum anti-lipid S IgG titres of patients with sternal SSI due to CoNS were significantly higher than the control patients ($P=0.001$). However, due to the small number of patients in this group, further evaluation of patients with SSI due to CoNS is required to confirm this trend. There was no correlation between the time post-surgically that the sternal SSI developed and the antibody titres in either the superficial or deep sternal SSI groups ($P=0.3$ and $P=0.4$ respectively).

The diagnostic parameters of the Lipid S ELISA are shown in table III.

Discussion

Sternal SSI is a serious condition with a significant associated morbidity and mortality. It is imperative that the diagnosis is established early so that treatment can commence. Several methods have been evaluated to diagnose sternal wound SSIs including; standard microbiological culture, sternal puncture,[21] infrared thermography of peristernal skin,[22] computed tomography, [6, 23] plain radiography [23] and radionucleotide imaging techniques using; Indium-111 labelled leukocytes,[6] Technetium-99m- labelled leukocytes [7] and monoclonal antigranulocyte antibodies.[7] However, all these current diagnostic techniques are often time consuming, expensive and lack sensitivity. The anti-staphylolysin test (ASTA) which is widely used in routine microbiology laboratories may aid in the diagnosis of SSI due to S. aureus, however the test has a poor sensitivity. Indeed, a study which evaluated the ASTA in patients with osteomyelitis due to S. aureus demonstrated on assay a sensitivity of 47% and specificity of 96%.[12]

The results of this current study highlight the potential of the lipid S ELISA as a rapid serological test to facilitate the diagnosis of sternal SSI due to both S. aureus and possibly CoNS. The test overall for S. aureus had a sensitivity of 68% and specificity of 97%, which compares well to previous studies which used this ELISA to diagnose central venous catheter-related infection,[13] endocarditis [16] and prosthetic joint infection.[17] The sensitivity of this test for S. aureus was higher in patients with deep sternal SSI (79%) compared to those with superficial sternal SSI (50%). This may be due to the chronic nature of deep SSI. Indeed, the sensitivity of the lipid S ELISA has been demonstrated to be higher in chronic infections such as endocarditis.[16]

Difficulties in determining whether or not CoNS are associated with sternal SSI or are sample contaminants remains a diagnostic challenge. The results in this study suggest

that the lipid S ELISA may aid in the diagnosis of true infection due to CoNS and may also provide a useful adjunct when interpreting cultures from patients with sternal SSI.

Approximately 30% of patients in this study with a clinical diagnosis of sternal SSI had no anti-lipid S IgG detected. There are several possibilities reasons for this observation. The time of onset of infection to collection of serum samples may not have been optimal to detect sufficient anti-lipid S IgG titres or the strain of S. aureus associated with the infection may have produced little or no exocellular lipid S antigen. These possibilities, in particular whether all staphylococcal strains produce lipid S and how anti-lipid S titres vary with time postoperatively in infected and non-infected individuals require further investigation.

In conclusion we present a sensitive and specific indirect ELISA for the rapid serodiagnosis of sternal SSI due to S. aureus which may aid in optimising patient management before culture results become available. The assay results from the five patients infected with CoNS also showed promising results. Further assessment of the lipid S ELISA for diagnosing these infections in particular those caused by CoNS is required in the clinical setting.

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Table I - Patient Demographics and Surgical procedures

	Control group (n=37)	Superficial sternal SSI due to S. aureus (CoNS)	Deep sternal SSI due to S. aureus (CoNS)
Mean age (years)	65	59 (67)	69 (65)
Age range (years)	43-82	48-74 (64-69)	51-80 (59-75)
Male	32	6 (2)	15 (3)
Female	5	6 (0)	4 (0)
Procedure: CABG	29	9 (2)	14 (3)
Valvular surgery	8	2 (0)	3 (0)
CABG and valvular surgery	0	1 (0)	1 (0)
Aortic surgery	0	0 (0)	1 (0)

CABG (Coronary Artery Bypass Graft)

Table II - Serum IgG titres to lipid S in patients with superficial or deep sternal SSI and non-infected controls.

Patient group		Superficial incisional SSI	Deep incisional SSI	Total in patient group
S.aureus sternal SSI	Total	12	19	31
	Number with positive IgG titres (%)	6 (50%)	15 (79%)	21 (68%)
	Mean anti-Lipid S IgG titre	7401	21068.3	15778
	Titre range	ND - 34479	ND - 51874	ND - 51874
CoNS sternal SSI	Total	2	3	5
	Number with positive IgG titres (%)	2 (100%)	3 (100%)	5 (100%)
	Mean anti-Lipid S IgG titre	2999	3163	3097
	Titre range	2243 - 3755	314 - 7372	314 - 7372
Non-infected patients	Total	-	-	37
	Number with positive IgG titres (%)	-	-	1 (3%)
	Mean anti-Lipid S IgG titre	-	-	251
	Titre range	-	-	ND - 9293

ND = non-detectable

Table III - The diagnostic parameters of the lipid S ELISA for sternal SSI due to S. aureus.

	Superficial sternal SSI	Deep sternal SSI	Total sternal SSI
Sensitivity	50%	79%	68%
Specificity	97%	97%	97%
Positive predictive value	86%	94%	95%
Negative predictive value	86%	90%	78%
Accuracy	86%	89%	84%

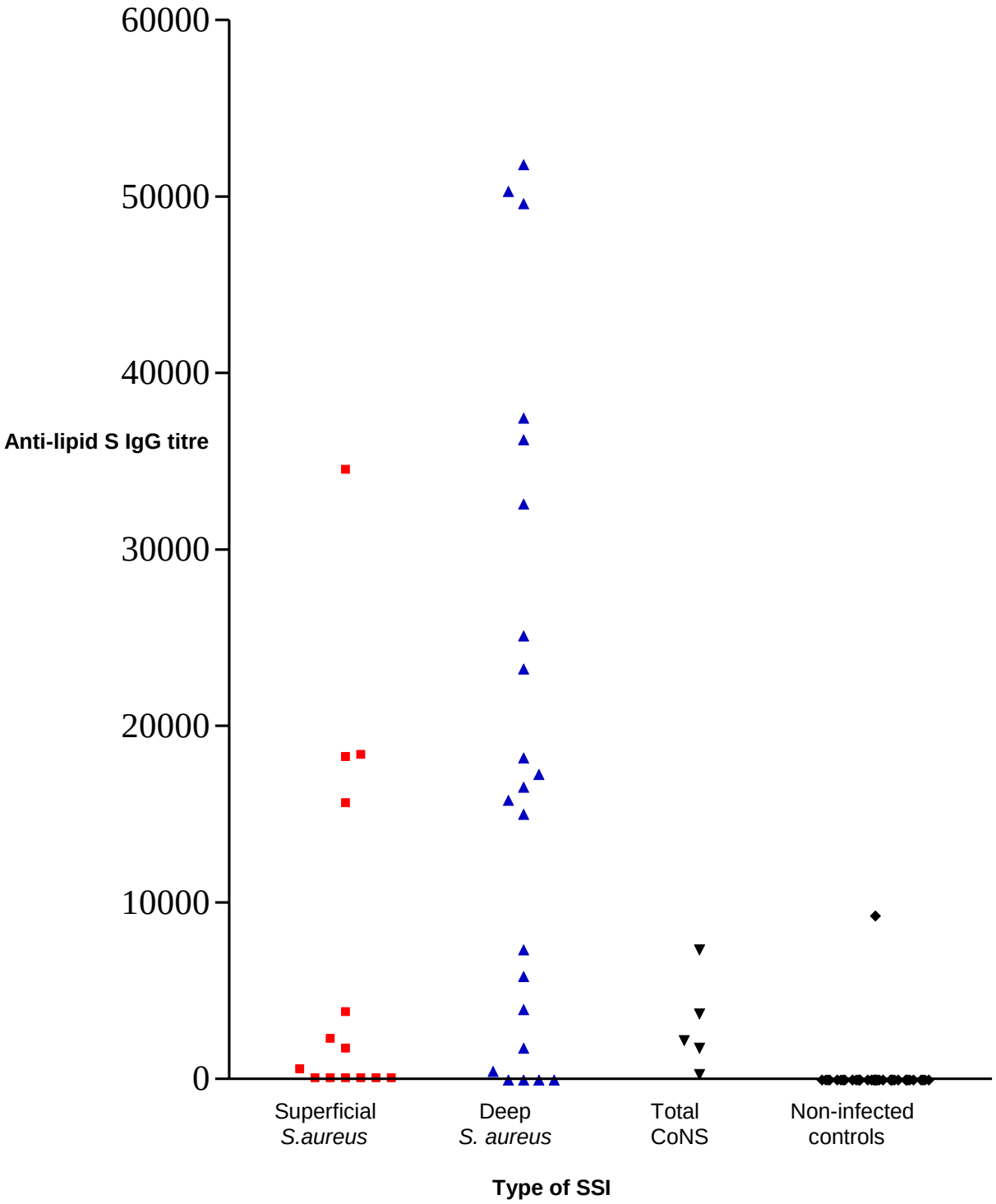

Figure 1 – Scattergram demonstrating the range of serum anti-lipid S IgG titres in patients with staphylococcal sternal SSI and controls.