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Clinical variables and *Staphylococcus aureus* virulence factors associated with venous thromboembolism in children

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ABSTRACT

Objectives: Children with *Staphylococcus aureus* (SA) bacteremia risk developing venous thromboembolism (VTE). We sought to identify clinical variables and bacterial virulence factors associated with VTE in SA bacteremia.

Study design: This is a single-institution retrospective study of 229 children with SA bacteremia hospitalized from 2005 to 2008. Clinical data were abstracted from patient charts. Two-hundred three SA isolates were analyzed by polymerase chain reaction. The Pediatric Health Information System (PHIS) database was queried to identify subjects with a central venous line (CVL) or complex chronic conditions (CCC). Logistic regression analysis was employed to determine which factors most greatly influenced VTE.

Results: VTE was present in 9.2% (n = 21/229). Superficial thrombi were excluded. Mortality was greater in patients with VTE [24% vs. 6% (p = 0.016)]. Among SA isolates available for virulence testing, the majority (70%; n = 139) were methicillin-sensitive SA (MSSA). Methicillin-resistant SA (MRSA) infection was associated with VTE (p = 0.01). The most common sites of thrombosis were extremity deep vein (58%; n = 14/24), head/neck (29%; n = 7), and visceral (13%; n = 3). One subject had a pulmonary embolism. The presence of a CVL or a CCC was not associated with VTE. Independent predictors of VTE were C-reactive protein (CRP) ≥ 20 mg/dl [OR 4.2, 95% CI 1.16–15.25] and hemoglobin nadir ≤9 g/dl [OR 5.2, 95% CI 1.3–20.64].

Conclusions: In addition to MRSA infection, CRP ≥ 20 mg/dl and hemoglobin nadir ≤9 g/dl were associated with VTE in SA bacteremia. These factors may serve as markers for increased risk of VTE with invasive SA disease.

1. Introduction

Venous thromboembolism (VTE) in children has been increasing in frequency, with an estimated incidence of 4.2 events/100,000 children per year. In hospitalized children, the incidence is higher at 58 events/10,000 hospitalizations per year [1–3]. The etiology of VTE in children is typically multifactorial, often involving underlying genetic predisposition [4,5]. Thrombosis usually occurs in the presence of a secondary risk factor, such as a central venous access catheter (CVL), cancer or infection/inflammation and children <1 year of age or those in adolescence are at higher risk [2–7].

Recently, attention has turned to prevention of VTE in hospitalized pediatric patients [3,8,9]. However, given the different underlying causes of VTE in pediatrics, data is lacking regarding the optimum approach to VTE prevention. Mere extrapolation of adult strategies for prevention are likely not relevant or appropriate for pediatric patients and thus, may not be successful. Therefore, current guidelines limit thromboprophylaxis recommendations to specific conditions, primarily based on expert opinion [10]. Current data also shows that thromboprophylaxis is inconsistently applied even among the highest risk patients [11,12]. Therefore, identifying specific clinical indicators for VTE risk within certain known high-risk scenarios may be a more successful strategy for early VTE detection and possible prevention in children.

Abbreviations: BSI, blood stream infection; *clfA*, clumping factor A; CRP, c reactive protein; CVL, central venous line; CCC, complex chronic conditions; *fnbA*, fibronectin binding protein A; *fnbB*, fibronectin binding protein B; LOS, length of hospital stay; MRSA, methicillin resistant *Staphylococcus aureus*; MSSA, methicillin sensitive *Staphylococcus aureus*; PCR, polymerase chain reaction; PHIS, Pediatric Health Information System; *pvl*, Panton–Valentine leukocidin; SA, *Staphylococcus aureus*; VTE, venous thromboembolism.

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Infection is a leading factor associated with VTE in children and *Staphylococcus aureus* (SA) is a commonly implicated bacteria resulting in severe infections leading to pediatric hospitalizations. There are few published clinical studies investigating the correlation between SA infections and the development of VTE. Only one previous study has systematically examined the correlation between VTE and the presence of SA virulence factors in a small group of pediatric patients with SA osteomyelitis [13]. In the current study, we evaluated the prevalence of VTE in patients with SA bacteremia at our institution from 2005 to 2008. We sought to identify clinical variables and/or bacterial virulence factors associated with the development of VTE in this population.

2. Patients and methods

2.1. Patients and S. aureus isolates

All blood cultures in which SA was the single species isolated from patients cared for at Children's Mercy Hospital (CMH) from January 1, 2005 through December 31, 2008 were identified by the CMH clinical microbiology laboratory and included in the study. All children ≤18 years admitted to our hospital during the specified time period with a single SA blood culture isolate were eligible for the study. Exclusion criteria included VTE present prior to development of SA bacteremia and presence of only superficial thrombosis. No patients were excluded for the first criterion and only 2 for the second. This study was approved by the Institutional Review Board at CMH. The STROBE guidelines were followed where they applied to the study [14].

Clinical data were abstracted retrospectively. Medical records were reviewed for the following information: date of birth, gender, date of hospital admission and discharge, anthropometric measurements, race, disposition, infection site(s), thrombosis site(s), imaging modality for thrombus identification, laboratory values (D-dimer, C reactive protein, white blood cell count, hemoglobin, platelet count, erythrocyte sedimentation rate), and coagulation studies (factor V Leiden mutation, prothrombin 20210 mutation, antithrombin activity, protein C activity, protein S activity, plasminogen level and factor VIII activity), if available. Subjects were identified as having a VTE if it was diagnosed on an imaging study obtained during the same hospitalization as that in which they had SA bacteremia, and subsequent to the diagnosis of SA. Diagnostic imaging modalities included ultrasonography with Doppler, computed tomography and magnetic resonance imaging. Additionally, the Pediatric Health Information System (PHIS) database was queried to identify complex chronic conditions (CCC) within the population and to determine which subjects had central venous lines (CVL) during the hospitalization in which SA bacteremia was identified. Central venous line insertion was determined either by ICD-9 code (38.93), procedure code (511161), or supply code (255130, 255131, 255132, 255133, 255134, 255135, 255136, and 255139) [15]. PHIS is an administrative database representing 44 freestanding children's hospitals affiliated with the Children's Hospital Association. Member hospitals contribute patient level data and rigorous data quality and reliability measures exist to ensure the integrity of the stored data.

2.2. DNA isolation

Genomic DNA was extracted from bacterial isolates by boiling with silicone beads. Several colonies from *S. aureus* cultures were collected into 0.8% saline solution and centrifuged. The bacterial pellet was then resuspended in 200 μL of 1× Tris–EDTA buffer. Sterile silicone beads were added to occupy 50% of the volume of bacterial suspension. The suspension was then vortexed for 5 min in a Disruptor Genie (Scientific Industries, Bohemia, NY) and the tube was placed in a 95 °C dry bath for 15 min. Following the incubation, the tube was centrifuged briefly to settle the beads and 100 μL of the supernatant containing the bacterial DNA was transferred to a new tube. DNA concentrations were measured using a Nanodrop-DN-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The DNA samples were stored at −20 °C until subsequent use.

2.3. Evaluation of S. aureus virulence factors

Polymerase chain reactions (PCRs) were performed for detection of the genes responsible for SA virulence factors listed in Table 1. The primer sequences, product length, and cycling conditions for each gene are given and have been previously described [16–21]. For *fnbB*, some isolates gave weak bands when viewed in a gel, so a second set of primers targeting an overlapping region of the gene was designed to resolve these. This primer set is listed in Table 1 as *Set 2*. Each PCR reaction also included an initial denaturation step at 95 °C for 5 min and a final extension step at 72 °C for 10 min. Amplifications were carried out on a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA) using Premix Ex Taq reagent mix (Clonetech Laboratories, Inc., Madison, WI) with a final concentration of 0.2 μM primers, except for *SCCmec* typing, where the primer concentrations varied. [21] *S. aureus* strains obtained from American Type Culture Collection Center (ATCC), ATCC #25904 and ATCC #25923, were used as positive controls in this study. The amplified PCR products were analyzed by 1% agarose gel electrophoresis.

2.4. Statistical analysis

Descriptive statistics such as medians, interquartile ranges and percentages were reported for demographic characteristics and presence or absence of thrombus. Laboratory variables were recorded as continuous variable whenever possible and as categorical variables when it was more clinically appropriate (for example, CRP). Each clinical variable was evaluated by bivariate analysis for association with the development of VTE. These tests included Wilcoxon Rank Sum, Chi-Square and Fisher's Exact tests. The association of each virulence factor with the development of VTE was examined by bivariate analysis including Chi-Square and Fisher's Exact tests.

Those variables reaching the predetermined level of significance ($p \le 0.1$) and those considered a priori to be clinically likely to be linked with VTE were included in a logistic regression model to determine which variables were most strongly associated with the development of VTE in patients with SA bacteremia. Odds ratios with 95% confidence intervals for development of VTE were determined. All statistical analysis was carried out using SAS software version 9.4 (Cary, NC, USA).

3. Results

Clinical characteristics of the 229 subjects with SA bacteremia are listed in Table 2. A total of 21 individuals were identified to have at least 1 VTE by imaging. There were 24 total venous thromboses, as some subjects had more than one VTE. The length of hospital stay (LOS) in days was significantly longer in the VTE group as compared to those without VTE (median LOS 19.5 days [IQR: 15–32.5] vs. 9 days [IQR: 5–20.5; $p < 0.01$). Mortality was also significantly higher in those with VTE [24% vs. 6% ($p = 0.016$)]. In the 5 subjects with VTE who expired, the cause of death in 3 was listed as MRSA sepsis with respiratory failure. Due to the retrospective nature of the data, the extent of contribution of thrombosis to each death was difficult to define. The other two subjects with VTE died of congenital heart disease with cardiopulmonary arrest. Two of the five were treated with anticoagulation. Of the 13 subjects without VTE who died, 5 succumbed to sepsis, 2 died from progressive oncologic disease, one died of progressive congenital heart disease and congestive heart failure, 3 from respiratory failure due to viral illness, one due to metabolic acidosis and one due to severe dehydration and shock. Of those 16 patients with VTE who survived, 13 were treated with anticoagulation. Resolution of the VTE was noted in 9 of these, two were noted to be persistent, and outcome was not recorded in two. No adverse events from anticoagulation were recorded.