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SHORT COMMUNICATION

Exfoliative toxin A staphylococcal scalded skin syndrome in preterm infants

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Abstract Staphylococcal scalded skin syndrome (SSSS) demonstrates dermal symptoms due to exfoliative toxin (ET) A or ETB produced by *Staphylococcus aureus*. We examined the association between anti-ETA antibodies and SSSS onset in neonates. Three preterm infants carried an ETA-producing strain of *S. aureus*, manifesting as either SSSS or bullous impetigo; a full-term infant carrying the same strain was asymptomatic. The infants (n=106) were categorized into three groups according to their gestational age (GA) as follows: <30 weeks, 30–37 weeks, and >37 weeks. The measured levels of anti-ETA antibody in the three infants displaying SSSS were low before the onset of dermal symptoms; only the asymptomatic full-term infant displayed a high antibody level. Anti-ETA antibody levels in the preterm group with a GA of <30 weeks were statistically lower than those in the term infant group; the prevalences of anti-ETA antibodies above a cutoff value in the three groups of neonates were 55 % (18/33) among preterm infants with a GA <30 weeks, 73 % (25/34) among those with a GA of 30–37 weeks, and 90 % (35/39) among infants with a GA >37 weeks. *Conclusion*: The presence of anti-ETA antibodies below a particular cutoff level might be associated with SSSS onset in preterm infants.

Keywords Staphylococcal scalded skin syndrome (SSSS) · Exfoliative toxin A (ETA) · *Staphylococcus aureus* · Methicillin-sensitive *S. aureus* (MSSA)

Abbreviations

BI	Bullous impetigo
ELISA	Enzyme-linked immunosorbent assay
ET	Exfoliative toxin
ELBW	Extremely low birth weight
GA	Gestational age
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NICU	Neonatal intensive care unit
OD	Optical density
SSSS	Staphylococcal scalded skin syndrome
VLBW	Very low birth weight

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Introduction

Staphylococcus aureus can cause exfoliative skin conditions, ranging from localized bullous impetigo (BI) to staphylococcal scalded skin syndrome (SSSS). SSSS generally affects infants and children between 6 months to 5 years of age [7, 10]; however, neonatal onset of SSSS, especially in low birth weight (LBW), preterm infants, has recently been reported [6, 8]. The symptoms of SSSS are caused by exfoliative toxin (ET) A or ETB. The relationship between SSSS onset and anti-ET antibody prevalence is unknown, especially in preterm infants. Our neonatal intensive care unit (NICU) experienced the horizontal transmission of a methicillin-sensitive *S. aureus* (MSSA) isolate, which produced ETA, and we examined the prevalence of anti-ETA antibodies among a neonatal population.

Case presentation

Case 1 was a preterm, very low birth weight (VLBW) infant (1,097 g) born at a gestational age (GA) of 28 weeks to a mother with a negative MSSA vaginal culture. We performed regular surveillance cultures on all NICU patients, and there had not been a known carrier of *S. aureus* in the NICU. At 13 days, the patient showed a 3-cm skin abrasion on the buttock and on the following day, another on the sole. Nikolsky's sign was positive, and the patient's forearm, back, buttocks, and face showed dermal symptoms, including redness and the subsequent development of abrasions (Fig. 1). Treatment with vancomycin (VCM) was started, and the dermal symptoms gradually improved and disappeared within approximately 2 weeks. Skin and pharyngeal cultures were positive for MSSA.

Case 2 was a preterm, extremely low birth weight (ELBW) infant (840 g) born at a GA of 27 weeks. One month after the onset of case 1, case 2 showed redness surrounding the umbilicus and MSSA was detected. Subsequent skin abrasions were seen in the skin over the jaw, upper arm, calf, axilla, and abdomen. Cefazolin was administered for 1 week, and the symptoms improved.

Case 3 was a preterm infant (2,212 g birth weight) born at a GA of 34 weeks. MSSA was first detected in the pharynx 1 month after the onset of case 1. A small amount of erythema and a crust were seen only around the umbilicus, but it did not spread to other parts of the body. Antibiotics were not administered, and the symptoms resolved within 5 days.

Case 4 was a full-term infant (2,056 g birth weight) born at a GA of 39 weeks. Two weeks after the onset of case 1, MSSA was detected in the infant's throat culture, but dermal symptoms did not develop.

A summary of the patients' characteristics is described in Table 1. The MSSA cultured from the four patients produced ETA, but not ETB, based on an EXT-RPLA Seiken assay (Denka Seiken, Tokyo, Japan). The MSSA strains were confirmed to be the same by random amplified polymorphic DNA and pulsed-field gel electrophoresis analyses.

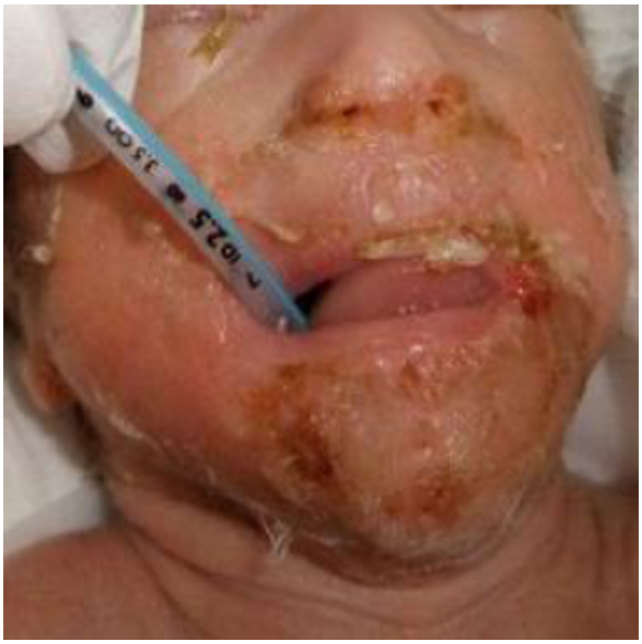

Fig. 1 Dermal symptoms in case 1; skin findings showed systemic decollement

Materials and methods

This study complied with the Helsinki Declaration, as revised in 2004, and was approved by the Ethics Committee of Shinshu University (approval no. 1666). Informed consents for this study were obtained from the parents of the patients.

Table 1 Summary of patient characteristics

	Case 1	Case 2	Case 3	Case 4
Gestation (weeks)	28	27	34	39
Birth weight (g)	1,097	840	2,212	2,056
Age at onset (days)	13	11	14	–
Symptom	SSSS	SSSS	Mild SSSS or BI	Asymptomatic
WBC (cells/μL)	11,970	7,900	10,760	–
CRP (mg/L)	0.02	0.23	0.01	–
Antibiotics	VCM	CEZ	not used	–
Serum IgG (mg/dL)	448	484	1,000	1,444

WBC white blood cells, *CRP* C-reactive protein, *IgG* immunoglobulin G, *BI* bullous impetigo, *SSSS* staphylococcal scalded skin syndrome, *VCM* vancomycin, *CEZ* cefazolin

ETA and serum samples

ETA was purchased from Toxin Technology (Sarasota, FL, USA). The purity was >95 %.

Serum samples from the four patients were collected before and after the onset of *S. aureus* infection. Serum samples were also collected from other infants born at our hospital during 2009 and 2010. Infants with severe complications, such as infections, respiratory diseases, chromosome aberrations, or clinical conditions requiring transfusions or intravenous immunoglobulin (IVIg) were excluded. The infants (n=106) were divided into three groups according to their GA: <30 weeks (n=33), 30–37 weeks (n=34), and >37 weeks (n=39). The serum samples were collected shortly after birth.

Enzyme-linked immunosorbent assay detection of antibodies to ETA

Briefly, 96-well enzyme-linked immunosorbent assay (ELISA) plates were coated with 100 μL of 1 mg/L recombinant ETA, serially diluted in phosphate buffered saline, and incubated overnight. After unbound ETA was removed, serum samples were added to the wells. Antigen-antibody complexes were detected by adding peroxidase-conjugated polyclonal antibodies against human IgG. The plates were developed by adding 100 μL of t-methylbenzidine and hydrogen peroxide to each well. The reaction was stopped by adding 0.5 mol/L H_2SO_4. The plates were read at 450 nm on a Spectra max PLUS384 (Molecular Devices, Sunnyvale, CA, USA) plate reader. Results were expressed as optical density (OD) values, with a blank subtracted.

Cutoff value determination

Because the prevalence of staphylococcal infections was unknown, we performed an ETA absorption test using the described ELISA method to determine a cutoff value for serum anti-ETA antibodies. Ten serum samples were chosen from term infants and reacted with various concentrations of ETA (0.05–2 mg/L). Significant difference was not seen in the absorbances of the 1–2 mg/L concentrations. The cutoff value was determined to be the absorbance at a concentration of 2 mg/L. The cutoff level (0.2742) reflected three standard deviations above the mean value (0.1379), with a standard deviation of 0.04543. OD values below the cutoff value were considered non-specific.

Statistical analysis

Statistical differences were determined using 2-tailed unpaired t tests. Differences with $p<0.05$ were considered significant.

Results

Anti-ETA antibody levels in SSSS patients

The anti-ETA antibody OD values were low in cases 1–3, and high in case 4 (Fig. 2a). All of the patients showed increased anti-ETA antibody levels a few months after the infection (Fig. 2b).

Anti-ETA antibody levels in term and preterm infants

The prevalences of anti-ETA antibodies in groups of neonates with GAs of <30 weeks, 30–37 weeks, and >37 weeks were 55 % (18/33), 73 % (25/34), and 90 % (35/39), respectively (Fig. 2c); the anti-ETA antibody levels of the preterm infants group with a GA of <30 weeks were statistically lower than those of the term infants group (p=0.0031). The preterm infants group with a GA of 30–37 weeks and the term infants group showed no significant difference (p=0.086).

Discussion

The MSSAs isolated from each of the four patients were identical clones that produced ETA and were horizontally transmitted throughout the NICU. In case 1, the route of infection was unclear, and the causative organism may have been brought into the NICU by a staff member or via another route from outside the NICU.

ET is known to be an exotoxin produced by *S. aureus*, and ETA was produced by the strain involved in our cases [7]. Although anti-ETA antibodies are known to neutralize the effects of ETA [4], there have been few quantitative studies, and the relationship between the onset of SSSS and the prevalence of anti-ET antibodies is unknown. This is the first report of the measurement of antibodies against ETA in preterm infants. In the current study, we referred to previous studies [13] and developed a specific ELISA to determine the distribution of anti-ETA antibodies.

Case 1–3 did not have anti-ETA antibody levels above the cutoff value prior to their infection; only in case 4 was the anti-ETA antibody level above the cutoff value (Fig. 2a). This might explain the onset of SSSS in cases 1–3 and the asymptomatic status of case 4. However, the severity of the disease varied among the symptomatic patients. The dermal symptoms were considerably milder in case 3 than in cases 1 and 2. Other factors, such as immunological immaturity and renal clearance of ET, may explain the disease severity differences.

SSSS and BI have been reported predominantly among infants and children under 5 years of age because individuals in this age group often lack specific anti-ETA and anti-ETB antibodies [2]. The likelihood of SSSS generally increases among children beginning at the age of 1 year, peaks at

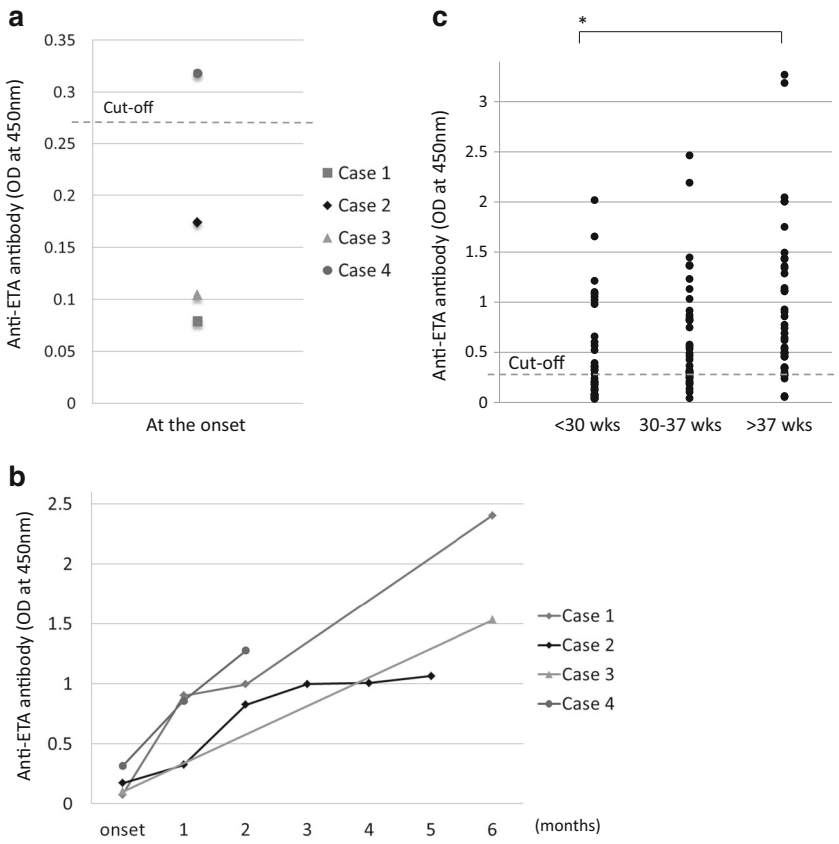

Fig. 2 **a** Anti-exfoliative toxin A (ETA) antibodies in the four patients prior to symptom development; only case 4 had an antibody level that was above the cutoff value. **b** Changes in the levels of anti-ETA antibodies in the four patients over time. **c** Anti-ETA antibody OD value comparisons between term and preterm infants. The anti-ETA antibody OD values and standard deviation in the groups at GAs <30 weeks, 30–37 weeks, and >37 weeks were 0.495±0.052, 0.638±0.489, and 0.971±0.762, respectively. The anti-ETA antibody levels are lower in preterm infants than in the full-term infants. The prevalence of the anti-ETA antibody levels above the cutoff value increased with increasing GA; *$p<0.01$

approximately 2–3 years, and declines to its lowest level among children aged 14–15 years. Maternal anti-ETA antibodies are thought to be passed to newborn babies; Melish et al. reported that approximately 88 % of newborns have anti-ETA antibodies in their umbilical blood [9]. This result was equivalent to the 90 % of term infants who demonstrated high levels of anti-ETA antibodies in our study (Fig. 2c). However, plasma IgG levels correlate with GA and, thus, anti-ETA antibody levels could be low in preterm or LBW infants [1]. In this study, the prevalence of anti-ETA antibody levels above the cutoff level in preterm infants with a GA <30 weeks was significantly lower than that in term infants. The development of SSSS in preterm infants is, therefore, postulated to be due to the low levels of antibodies against the toxins [11]. However, as previously mentioned, there are many other reasons that may explain why case 4 did not develop SSSS despite a positive staphylococcal culture. These include a more potent natural antibacterial immunity in term infants as well as the presence of various kinds of anti-staphylococcal antibodies, except for those specific for ETA [3]. Thus, explaining the onset of SSSS, based only on anti-ETA antibody levels, may be difficult.

SSSS in neonates is typically mild and generally treated with antibiotics [7, 10]. However, because of antimicrobial resistance in clinical strains of *S. aureus*, antibiotics are not always effective and a few fatal cases, including ones requiring plasmapheresis have been reported [5, 12]. SSSS symptoms are mainly caused by ETA or ETB, and antibodies against these toxins have been demonstrated to neutralize their effects [4]. Thus, the administration of IVIg as a treatment modality has also been reported [10, 6]. Commercial immunoglobulin is concentrated and refined from the sera of 10,000–20,000 individuals and contains high levels of anti-ETA antibodies [13]. We confirmed a great deal of anti-ETA antibody was present in the immunoglobulin in this study (data not shown), supporting the use of IVIg as an option for the effective treatment of SSSS.

Conclusion

Anti-ETA antibody levels were significantly lower among patients demonstrating SSSS and also among preterm infants compared with healthy, full-term infants. The presence of low levels of anti-ETA antibodies might be associated with the onset of SSSS.

Conflict of interest The authors declare that they have no conflicts of interest to report.

Financial disclosure The authors have no financial relationships relevant to this article to disclose.

References

1. Ballow M, Cates KL, Rowe JC, Goetz C, Desbonnet C (1986) Development of the immune system in very low birth weight (less than 1500 g) premature infants: concentrations of plasma immunoglobulins and patterns of infections. Pediatr Res 20:899–904
2. Cribier B, Piemont Y, Grosshans E (1994) Staphylococcal scalded skin syndrome in adults. A clinical review illustrated with a new case. J Am Acad Dermatol 30:319–324
3. Dryla A, Prustomersky S, Gelbmann D, Hanner M, Bettinger E, Kocsis B, Kustos T, Henics T, Meinke A, Nagy E (2005) Comparison of antibody repertoires against *Staphylococcus aureus* in healthy individuals and in acutely infected patients. Clin Diagn Lab Immunol 12:387–398
4. Gemmell CG (1995) Staphylococcal scalded skin syndrome. J Med Microbiol 43:318–327
5. Hoffmann R, Lohner M, Bohm N, Schaefer HE, Leititis J (1994) Staphylococcal scalded skin syndrome (SSSS) and consecutive septicaemia in a preterm infant. Pathol Res Pract 190:77–81
6. Kapoor V, Travade J, Braye S (2008) Staphylococcal scalded skin syndrome in an extremely premature neonate: a case report with a brief review of literature. J Paediatr Child Health 44:374–376
7. Ladhani S, Joannou CL, Lochrie DP, Evans RW, Poston SM (1999) Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome. Clin Microbiol Rev 12:224–242
8. Makhoul IR, Kassis I, Hashman N, Sujov P (2001) Staphylococcal scalded-skin syndrome in a very low birth weight premature infant. Pediatr 108:E16
9. Melish ME, Chen FS, Sprouse S, Stuckey M, Murata MS (1981) Epidermolytic toxin in staphylococcal infection: toxin levels and host response. Zentralbl Bakteriol 10(Suppl):287–298
10. Patel GK (2004) Treatment of staphylococcal scalded skin syndrome. Expert Rev Anti Infect Ther 2:575–587
11. Rieger-Fackeldey E, Plano LR, Kramer A, Schulze A (2002) Staphylococcal scalded skin syndrome related to an exfoliative toxin A- and B-producing strain in preterm infants. Eur J Pediatr 161:649–652
12. Tenenbaum T, Hoehn T, Hadzik B, Stammogel H, MacKenzie CR, Mayatepek E, Schroten H (2007) Exchange transfusion in a preterm infant with hyperbilirubinemia, staphylococcal scalded skin syndrome (SSSS) and sepsis. Eur J Pediatr 166:733–735
13. Yamasaki O, Yamaguchi T, Sugai M, Chapuis-Cellier C, Arnaud F, Vandenesch F, Etienne J, Lina G (2005) Clinical manifestations of staphylococcal scalded-skin syndrome depend on serotypes of exfoliative toxins. J Clin Microbiol 43:1890–1893