Toxic shock syndrome in the burned patient

VALERIE EDWARDS-JONES and SUSAN G. SHAWCROSS
Department of Biological Sciences, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, England, UK
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Abstract: Toxic shock syndrome (TSS) has gained notoriety because of its association with tampon use. However, there is an increasing awareness of the syndrome on many of the specialised burn units in hospitals throughout the United Kingdom. TSS primarily affects children with small-percentage burns, and it is this group of patients that normally would be expected to make an uneventful recovery. One unit, where 100–150 children are admitted per year, has seen four cases of confirmed TSS over a two-year period. There does not appear to be the same risk of TSS in adult burned patients, and this lower incidence may be the result of an increase in the production of antibodies to toxic shock toxins with increase in age.

Key words: Burns. Shock, septic. Staphylococcus aureus.

Introduction

Toxic shock syndrome (TSS) was first described in 1978 by Todd et al. in seven children who presented with similar symptoms (fever, vomiting, diarrhoea, rash, disseminated intravascular coagulation [DIC]), which led to shock and multi-organ failure. One child died and the remainder showed desquamation of the skin on their hands and feet during convalescence. In five of the seven cases, Staphylococcus aureus phage group I was isolated from various focal lesions. Subsequently, it was shown that a toxin was involved in the pathogenesis of the syndrome. This toxin was initially thought to be an enterotoxin because of its emetic effect on monkeys, thus it was named staphylococcal enterotoxin F. Other workers showed that it had a pyogenic effect on rabbits, and the toxin was designated pyrogenic exotoxin C. Later biochemical studies showed that the two toxins were identical and it was renamed toxic shock syndrome toxin 1 (TSST-1). A strict definition of TSS was devised by the Centers for Disease Control (CDC) in Atlanta and this allowed cases to be defined for epidemiological purposes (Table 1).

| Table 1. Classic criteria for clinical diagnosis of TSS
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<tr>
<td>Fever above 38.9°C</td>
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<td>Hypotension or orthostatic dizziness</td>
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<td>Diffuse or palmar erythema</td>
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<tr>
<td>Desquamation of hands and feet</td>
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<td>Hyperaemia of conjunctiva and of the mucous membranes of the oropharynx or vagina</td>
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<td>Multi-system dysfunction which must include at least four of the following:</td>
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<td>Diarrhoea and vomiting</td>
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<td>Alterations in consciousness</td>
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<td>Impaired renal function</td>
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<td>Impaired hepatic function</td>
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<td>Thrombocytopenia</td>
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<td>Elevated muscle creatine phosphokinase</td>
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<tr>
<td>Cardiopulmonary dysfunction</td>
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<td>Decreased serum calcium and phosphate</td>
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Epidemiology

By the early 1980s, 941 cases of TSS had been reported to the CDC. It was found that 95% of cases occurred in previously healthy women, during or soon after a menstrual period—menstrual TSS (MTSS). Detailed epidemiological studies showed a link between MTSS and tampon use. More specifically, there appeared to be a direct link between changes made to the tampon material composition by the manufacturers and an upsurge in the number of cases of MTSS. During the mid- to late 1970s, carboxymethylcellulose materials were added to tampons to increase their absorbency. Subsequently, these materials were shown to induce an increase in the production of TSST-1 by Staphylococcus aureus. These materials were removed from the manufacturing process in 1985,
and the incidence of MTSS has fallen dramatically since.\textsuperscript{13}

The reported incidence of non-menstrual TSS (NMTSS) increased as the clinical symptoms were recognised. Between 1978 and 1982 there was a four-fold increase in the number of cases reported, and by 1987, 20–30% of reported cases of TSS in the USA were of the non-menstrual type.\textsuperscript{14} At present in the UK there are about 40 cases of TSS reported per year, 50% of which are NMTSS.\textsuperscript{15} NMTSS occurs in men, women and children, and is associated with a plethora of localised \textit{S. aureus} infections involving surgical wounds, post-therapeutic abortions, deep abscesses, lacerations, furuncles,\textsuperscript{16} pyomyositis\textsuperscript{17} and rhinoplasty.\textsuperscript{18} In recent years reports have linked staphylococcal enterotoxins and TSST-1 toxin with sudden infant death (SIDS) syndrome.\textsuperscript{19} The first cases of TSS in burned children were reported in 1985 by Frame \textit{et al.},\textsuperscript{20} and these were followed quickly by several other reports.

\section*{Toxins associated with TSS}

More than 90% of MTSS and 60% of NMTSS is caused by TSST-1 toxin from \textit{S. aureus}.\textsuperscript{21} Many cases of TSS, not attributable to TSST-1, are caused by the other staphylococcal toxins. The staphylococcal enterotoxins A (SEA), B (SEB) and C (SEC),\textsuperscript{22} and exfoliative toxins also have been associated with TSS.\textsuperscript{21,23} SEB is the most common staphylococcal enterotoxin to be associated with NMTSS.\textsuperscript{15} Studies of the organism showed that MTSS is caused by a single clone of \textit{S. aureus} which produces both SEA and TSST-1, and NMTSS is caused by a variety of strains that usually produce TSST-1 as a single toxin.\textsuperscript{24}

Strains of \textit{S. aureus} associated with TSS have the following characteristics: decreased haemolysis of rabbit RBCs; an absence of plasmids; decreased arsenate susceptibility; increased bacteriocin susceptibility; production of SEA, B, C, D or E; production of pigment; and, an increase in casein proteolysis.\textsuperscript{2} The strains produce gamma lysin in preference to alpha lysin,\textsuperscript{25} are tryptophan-dependent (80% MTSS, 40% NMTSS)\textsuperscript{26} and usually possess a type-8 capsule.\textsuperscript{27} Resistance to penicillin, cadmium and arsenate occurs frequently in TSST-1-producing strains, and all strains are sensitive to mercury.\textsuperscript{28,29}

Streptococcal pyogenic toxins A, B or C, which are produced by \textit{Streptococcus pyogenes}, cause a condition very similar to TSS called toxic shock-like syndrome (TSLS).\textsuperscript{30} However, unlike TSS, fasciitis and/or myositis frequently develop with this disease.\textsuperscript{31} Some of the toxins produced by \textit{Pseudomonas aeruginosa} have been associated with the development of TSS, albeit infrequently,\textsuperscript{32} and endotoxins produced by Gram-negative bacteria have been shown to potentiate the effect of the staphylococcal toxins \textit{in vivo}.\textsuperscript{13}

\section*{Regulation of toxin production}

TSST-1, enterotoxins, haemolysins, leucocidins, surface proteins (\textit{e.g.} protein A) and various enzymes (\textit{e.g.} coagulase) produced by \textit{S. aureus} are regulated globally by the accessory gene regulator (\textit{agr}). This acts by enhancing the transcription of target genes or by stabilising the transcripts.\textsuperscript{34,35} The \textit{agr} locus has been isolated and sequenced,\textsuperscript{36} and consists of two divergent transcription units driven by the promoters P2 and P3. The promoters are regulated temporally: they are weakly active in the early exponential phase and strongly active later in the growth cycle.\textsuperscript{37,38} A third promoter, P1, is weakly constitutive, but, as yet, its function is unknown.\textsuperscript{36} The P2 transcript includes a four-gene operon, \textit{agrA}, \textit{agrB}, \textit{agrC} and \textit{agrD}, which forms an autocatalytic circuit, all components of which are required for an \textit{agr} response. \textit{agrA} corresponds to the response regulator of a classical two-component sensory transducer system, and \textit{agrB} to a histidine phosphokinase sensory transducer.\textsuperscript{39} Both of these components may sense external metabolite status. The \textit{agr} regulon is auto-regulated and is induced by a protein secreted by bacterial cells.\textsuperscript{40} RNAI and RNAII are produced by this four-gene operon.\textsuperscript{39} The second transcription unit, which uses promoter P3, specifies a 517-nucleotide transcript, RNA III, which is required for exoprotein regulation. RNA III also encodes the 26-amino-acid residue, delta haemolysin; however, this is not responsible for \textit{agr} regulation.\textsuperscript{37-39} RNAIII activates transcription of secretory proteins at the end of exponential growth and suppresses the transcription of surface proteins \textit{via RNA II}.\textsuperscript{57} Two further polycistrionic regulatory loci have been identified: the staphylococcal accessory regulator (\textit{sar})\textsuperscript{41} and the extracellular protein regulator (\textit{spr}).\textsuperscript{42} These contribute to the regulation of a similar set of exoproteins and enzymes; \textit{sar} also complements the effect of \textit{agr}.\textsuperscript{41}

\section*{Expression of TSST-1 toxin}

Expression of TSST-1 and many other exotoxins occurs at the end of the exponential growth phase. Optimal conditions for the production of TSST-1 include an aerobic environment, neutral pH, 6% carbon dioxide, temperature of 40°C, and the presence of proteins, plasma and serum,\textsuperscript{43-45} which can all be found in infectious foci\textsuperscript{44}—including burn wounds. Low concentrations of metal ions, especially magnesium, have been shown to affect TSST-1 production. In MTSS it has been proposed that magnesium binding by certain tampons can lead to magnesium limitation, which in turn increases TSST-1 production.\textsuperscript{46,47} This may also occur at the burn wound: dressings and/or antiseptic creams may interfere with toxin production by a similar mechanism. Flamazine\textsuperscript{43} cream, which contains 1% silver sulphadiazine, is used in 77% of burn units.\textsuperscript{48} This compound has
been shown to enhance TSST-1 production at least fourfold in 45% of the S. aureus strains tested \textit{in vitro}.\textsuperscript{49} It is not clear whether these data are representative of \textit{in vivo} systems as studies using animal models have not yet been performed.

\section*{Structure of the toxins}

A comparison of the amino acid sequences of toxins involved in TSS places them into four distinct groups. These groups comprise: i) SEA,SED and SEE; ii) SEB, SECl-C3, SPE-A and SPE-C; iii) TSST-1 toxin; and iv) exfoliative toxins A and B and SPE-B.\textsuperscript{21} The TSST-1 toxin and the staphylococcal toxins are single-chain polypeptides with molecular weights ranging from 22 to 29 kDa.\textsuperscript{5,22} TSST-1 molecules do not have the cysteine loop,\textsuperscript{23} which is a characteristic of enterotoxins. TSST-1 has little primary sequence homology with the enterotoxins; however, there are striking similarities between the tertiary structures of TSST-1 and SEB. The structures of these toxins have been determined by X-ray crystallography, and are composed largely of $\beta$ strands within two distinct domains.\textsuperscript{50,51} The tertiary structure of TSST-1 and SEB gives the toxins their biological activity in the development of TSS. The staphylococcal enterotoxins, TSST-1 and streptococcal pyogenic toxins (A–C) collectively are called superantigens (SAGs) because of their profound effect on the immune system and, ultimately, the development of TSS.\textsuperscript{52}

\section*{Superantigens}

The mechanism of T-cell stimulation by SAGs, such as TSST-1, is unique. The SAGs cross-link the variable $\beta$ region on the T-cell receptor (TCR) with major histocompatibility complex (MHC) II molecules on the target cell (Fig. 1a). The normal function of MHC II molecules is to present processed antigen, via the inner groove of the molecule, to T-cells (Fig. 1b). However, SAGs bind to the MHC II on the outer groove of the molecule, without being first processed. SAG-MHC II complexes are presented to, and stimulate, T-cells which bear specific beta chain (V$\beta$) regions.\textsuperscript{52,53} For example, TSST-1 stimulates T-cells which have a V$\beta$2 region.\textsuperscript{54} Some 5–30% of the T-cell population can be stimulated by a superantigen, whereas a conventional antigen will only stimulate one in 10000 cells.\textsuperscript{55} It has been suggested that activation of antigen-presenting cells and T-cells produces large amounts of tumour necrosis factor (TNF),\textsuperscript{56} interleukin-1 (IL-1),\textsuperscript{57} interleukin-6 (IL-6) and interferon $\gamma$, and it appears to be the over-activation of these cytokines that produces some of the clinical symptoms seen in TSS.\textsuperscript{58}

\section*{Toxic shock in the burned patient}

\subsection*{The burn and its clinical management}

When a burned patient is admitted to hospital the immediate priority is to prevent shock and excessive

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\textbf{Fig. 1.} (a) The interaction of a superantigen (SAg), T-cell receptor (TCR) and major histocompatibility molecule (MHC) on an antigen-presenting cell (APC). Superantigens bind to the outer regions of both the MHC molecule and the V$\beta$ region of the T-cell receptor. (b) Binding of a conventional antigen (Ag) within the groove formed by the $\alpha$ and $\beta$ chains of the MHC molecule.
fluid loss. Once the patient has stabilised, attention is then focused on the wound. Wound management procedures for the burned patient differ between burn units, but the majority use ointments and/or impregnated dressings in an attempt to prevent burn-wound infection.58

A burn wound results in the loss of a physical barrier to infection, and is accompanied by an inflammatory response. Within a few minutes oedema develops beneath the affected areas as a result of changes in capillary permeability caused by the tissue damage. Blisters, dead epithelium and burned clothing are removed, and the wounds are cleaned and then assessed for treatment. Surface burns are classified by the extent of injury, defined as a percentage of the total body surface area (TBSA) involved, and the depth of the injury. The source of the burn (e.g. scald, flame burn, corrosive chemical burn or flash explosion) is of less importance than the depth and extent of injury. The depth of the burn is estimated by its initial physical appearance and by continued monitoring of appearance over several days. It is important to estimate quickly the extent of injury so that appropriate treatment can be given, in particular intravenous fluid therapy.59

Dressings are used to cover the wound and absorb plasma leakage. The ideal burn covering should provide adhesion, water vapour transport, elasticity and an intact bacterial barrier. The dressings are changed regularly (e.g. every 24–48 h) and the wound is monitored continually for signs of infection.50 The procedure in many burn units is to debride the wound as soon as possible and graft with the patient’s own skin from an unaffected site on the body. If no suitable sites remain on the patient’s body then artificial skin coverings are used. This renders the wound less susceptible to infection.60 Antibiotic prophylaxis is not part of the normal management of burns.62

Patients with more than 10% TBSA burns are usually infused with intravenous fluids or plasma expanders to replace fluid losses.63 Patients with less than 10% TBSA burns do not generally receive fluid resuscitation unless it is clinically indicated: their treatment focuses primarily on wound management, but this is dependent upon the nature of the burn. Blood is often given when the burn eschar is excised, and replaces fluid lost during the operation. It is the patient with less than 10% TBSA burns who has the highest risk of developing TSS,20 and this is probably due to a lack of specific antibodies – either passive or acquired.

The immune status of the burned patient

In severely burned patients the immune system becomes profoundly impaired. Serum immunoglobulin and complement levels are decreased, chemotaxis of neutrophils is depressed and cell-mediated immunity is diminished. The aetiology of the response is not understood fully but is thought to be related to the massive inflammation caused by the burn wound, and to protein loss from the skin surface. Development of anaemia is a characteristic of severely burned patients.64

In some UK burn units, several cases of TSS have been reported in burned children,65–67 some of which have resulted in death.20 In a typical case of TSS the patient will present with severe pyrexia (40°C), tachycardia (200 beats/min), and tachypnoea (51 breaths/minute) quite soon after admission (mean: 2.2 days). This progresses rapidly to include vomiting, diarrhoea, rash and oliguria. Some may also show neurological disturbances.67 Specific treatment against the toxin involves the use of passive antibodies present in intravenous human immunoglobulin, fresh frozen plasma or whole blood,67 75% of which have been shown to contain antibodies to TSST-1.68 These antibodies protect the patient against the effects of the toxin. Fluclaxacinil is administered to destroy S. aureus and prevent further release of toxin. The effects of hypovolaemic shock are treated with blood or plasma expanders.67

Why do burned patients develop TSS?

Several factors must be present for the burned patient to develop TSS.

1. The patient must be susceptible to the effects of the superantigenic toxin.
2. The burn wound becomes colonised or infected with toxin-producing bacteria.
3. The environment of the wound must allow the bacteria to grow and produce toxin.

Host susceptibility

Serological studies by Vergeront et al.69 showed that antibodies to TSST-1 develop with age. Studies show that 47%, 58%, 70%, 88%, 96% and 99% of the population are antibody-positive at the age of one, five, 10, 20, 30 and 50 years respectively. The occurrence of antibodies to the other enterotoxins (e.g. SEA, SEB, etc.) varies within the population.70

In general, children admitted to burn units in the United Kingdom are aged between 18 months and three years.48 An investigation into the presence or absence of antibodies to TSST-1 in children admitted to a paediatric burn unit in Manchester showed that 49% possessed antibodies to TSST-1.68 These data agree with the incidence of childhood TSST-1 antibodies reported by Vergeront et al.69

Antibodies to TSST-1 are usually absent from the sera of patients with TSS, in contrast to age-matched control subjects from the population.71 Patients who suffer recurrent TSS do not produce antibodies to either TSST-1 or other enterotoxins.71 TSST-1 and enterotoxin antibodies

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were shown to protect against and prevent the development of TSS in animal models given passive immunisation with TSST-1 monoclonal antibodies. These results indicate that individuals are susceptible to TSS because they lack TSST-1 antibody. However, antibody production status is not the only important factor in TSS development. Studies by Jacobson et al. showed that patients infected with a TSST-1-producing strain of *S. aureus*, who also lacked antibodies to TSST-1, did not develop TSS. This was later substantiated by Childs et al. in a prospective study of burned children. Therefore, the inherent susceptibility (i.e. the TcR and MHC type of the patient) to the superantigenic effect of the toxin is a contributory factor in the development of TSS. The proportion of the population possessing the appropriate MHC II and TcR type has yet to be determined.

Burn wound colonisation

Burn wound infection is the primary cause of morbidity and mortality in burns patients. The injury disrupts both the normal skin barrier and many of the systemic host defence mechanisms that prevent infection. Immediately following injury the wound is sterile, but within a few hours it is colonised by a variety of microorganisms. The resulting eschar provides an excellent growth medium for many microorganisms. Damage to the vascular supply impedes the efficacy of the immune defence mechanisms. Further, the poor vascularisation of the wound restricts the delivery, and, therefore, reduces the effectiveness of systemic antibiotics. Burn wounds are susceptible to colonisation and subsequent infection by a multitude of microorganisms, including the normal body flora, however, eventually pathogenic microorganisms predominate. Prior to the widespread use of topical antimicrobial agents, many patients developed Gram-negative septicaemia or serious *S. pyogenes* infections and died. At this time, *S. aureus* is the most frequently isolated pathogen found in a burn unit. The increased occurrence of *S. aureus* is a result of the prophylactic use of agents, and changes in wound management procedures. On most burn units the wound is debrided as soon as possible, then grafted. The early closure of the wound reduces the risk of serious infection. There are now very few cases of Gram-negative septicaemia, and any variations seen are probably due to differences in local practices. Colonisation rate of the burn wound varies between individual burn units, and can be as high as 30%. Infection of wounds other than burns (e.g. surgery or trauma) occurs in about 5% of cases. Eleven per cent of burn wounds are colonised by microorganisms within one day of injury; the most common being *S. aureus*, with an incidence of 50%. *S. pyogenes* and *P. aeruginosa* often infect the wound, and can cause major problems such as graft failure and wound separation. These microorganisms have also been implicated as a possible cause of TSS.

Toxins SEA, B, C or D and TSST-1 are produced by at least 50% of *S. aureus* isolated from burned patients, and similar results may be found in non-burned patient groups. Sixty per cent of the patients colonised with *S. aureus* carry toxin-producing strains, and one in five patients carries mixed strains (toxin- and non-toxin-producing) within the wound. Investigation of the colonisation patterns of *S. aureus* shows that in many cases the same phage type is isolated from successive swabs. In some patients, new phage types appeared but the original strain usually remained. Recent work by our group at the Manchester Metropolitan University showed that when a TSST-1- and/or SEA-producing strain of *S. aureus* was grown in mixed culture with a different toxin-producing strain (SEB or SEC) or a non-toxin-producing strain, TSST-1 and SEA production by the former strain increased. Further, it was found that the increase in toxin production was dependent on inoculum size (Sargent et al., unpublished data). It is not known whether this occurs at wound sites, but the system merits further investigation. It is possible that the effect described is similar to the phenomenon of quorum sensing. Work by some groups (e.g. Balaban and Novick and Jet et al.) showed that the synthesis of virulence factors by *S. aureus* is controlled by an autotrophic cell density detection system that utilises an octapeptide secreted by the microorganism. The secreted octapeptide activates the agr regulon – the global regulator of the virulence response. These preliminary findings on mixed culture stress the importance of a comprehensive microbial screening programme when monitoring burn wounds, especially in patients who may have TSS.

The wound environment

A burn wound is moist, often due to draining lymph or plasma. Both are rich in nutrients, especially proteins, and this favours bacterial growth. The composition of burn oedema is similar to that of plasma but has a lower protein content. The physical environment of the wound, including the availability of oxygen, the presence of immune factors and the nutritional status, will depend upon the patient and the nature of the burn.

Factors influencing toxin production at the burn wound

Physical factors, such as oxygen availability, pH of the surrounding fluid and availability of trace metal ions, might create a favourable environment for toxin production. Tissue trauma may result in the release of endogenous proteases into the burn wound; these may affect toxin production. The presence of both blood and protein has been shown to increase the amount of toxin produced by *S. aureus*. Burn dressing components
have been implicated as possible environmental factors that stimulate toxin production; however, this has not been substantiated in the laboratory. All reports of TSS associated with burns state that the wounds had been dressed, and it is possible, therefore, that some burn dressings provide conditions that are favourable for toxin production. Perhaps some dressing component(s) chelate magnesium and thereby increase toxin production. Some highly absorbent burn dressings contain carboxymethylcellulose which was associated with an increase in the number of cases of MTSS. Antimicrobial agents applied routinely to wounds to prevent infection have been shown to increase TSST-1 production by some strains of *S. aureus* in *vitro*. What, if any, effect the application of more than one antimicrobial agent to a wound has on toxin production is not known. Further investigation of the role of dressings and topical antimicrobial therapy in the development of TSS is required. In addition, a suitable model that mimics the situation in *vivo* is needed.

**Conclusion**

Early diagnosis of TSS in burned patients is essential because the mortality rate may be as high as 50%. In addition, early intervention and the administration of specific antibiotics are crucial to the management of TSS. However, the single most important therapeutic measure is the administration of pre-formed anti-TSST-1 antibodies as human immunoglobulin, fresh frozen plasma or fresh blood, which results in a state of passive immunity in the recipient. In 1993, Takei et al. showed that antibodies function by inhibiting the binding and/or presentation of staphylococcal toxins to cells displaying MHC II.

The number of burned patients with TSS seems to have increased since the early 1980s, and may be attributable to changes in patient management. In addition to changes in wound management, the compositions of resuscitation fluids have changed. The use of high plasma protein fraction (HPPF), which is a colloid and does not contain immunoglobulins, has superseded the use of fresh frozen plasma (FFP), which contains immunoglobulins.

In 1990, Cole and Shakespeare recommended that the diagnostic criteria of TSS in burned patients should be simplified. The recommended criteria were: pyrexia (39 °C body temperature), rash, shock, diarrhoea and/or vomiting, irritability, lymphopenia. Clinical intervention was recommended when these criteria were fulfilled in an attempt to prevent the full clinical picture developing, and to reduce the probability of death. Unfortunately, these symptoms are similar to those seen at the onset of many other infectious diseases. One consequence of this ambiguity may be an over-diagnosis of TSS by some units. Many suspected cases of TSS remain unconfirmed because, due to early clinical intervention, the diagnostic criteria for TSS were not fulfilled.

Why TSS develops in some burned children is not understood fully, but as research progresses, many risk factors may be highlighted. Ongoing investigation of all of these factors may lead eventually to the prevention of TSS in burned patients.

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