MAJOR ARTICLE

# Intravenous Immunoglobulin G Therapy in Streptococcal Toxic Shock Syndrome: A European Randomized, Double-Blind, Placebo-Controlled Trial

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**(See the Editorial Commentary by Stevens on pages 341–3)**

**The efficacy and safety of high-dose intravenous polyspecific immunoglobulin G (IVIG) as adjunctive therapy in streptococcal toxic shock syndrome (STSS) were evaluated in a multicenter, randomized, double-blind, placebo-controlled trial. The trial was prematurely terminated because of slow patient recruitment, and results were obtained from 21 enrolled patients (10 IVIG recipients and 11 placebo recipients). The primary end point was mortality at 28 days, and a 3.6-fold higher mortality rate was found in the placebo group. A significant decrease in the sepsis-related organ failure assessment score at days 2 ($P = .02$) and 3 ($P = .04$) was noted in the IVIG group. Furthermore, a significant increase in plasma neutralizing activity against superantigens expressed by autologous isolates was noted in the IVIG group after treatment ($P = .03$). Although statistical significance was not reached in the primary end point, the trial provides further support for IVIG as an efficacious adjunctive therapy in STSS.**

The 2 most severe manifestations of invasive infections caused by group A streptococci (GAS) are necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). The latter is characterized by hypotension and multiorgan failure early in the course of infection [1]. GAS express several virulence factors, both cell bound and secreted, which have been shown to be important in pathogenesis. A pivotal role for superantigens in mediating the systemic effects of STSS has been shown [2, 3]. Previous studies have shown that protective humoral immunity to both cell-associated and soluble GAS virulence factors are important in preventing invasive disease [4–8]. Patients with invasive GAS disease had significantly lower serum levels of protective antibodies against M-protein and superantigens, compared with serum samples from noninvasive cases [4–7]. These findings provided evidence that lack of protective humoral immunity against the relevant GAS virulence factors contributes to susceptibility to invasive infection.

There is a definite need for adjunctive therapy in severe invasive GAS infections because the mortality rate can be as high as 80% despite prompt antimicrobial

Received 9 January 2003; accepted 20 March 2003; electronically published 17 July 2003.

Financial support: Baxter Healthcare; Swedish Foundation for Strategic Research and the Swedish Research Council (to J.A. and A.N.-T.).

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**Clinical Infectious Diseases 2003; 37:333–40**

1058-4838/2003/3703-0003$15.00

therapy in many cases [9]. Previous studies on humoral immunity in invasive GAS disease have highlighted the importance of antibodies in protection against these infections, and they suggest that immunoglobulins might be a useful adjunctive therapy. Human polyspecific intravenous IgG (IVIG) was suggested as a potential adjunctive therapy for invasive GAS diseases mainly because of its ability to neutralize a wide variety of superantigens and to facilitate opsonization of streptococci [10]. An observational cohort study of IVIG therapy involving patients with STSS reported decreased mortality rates in patients treated with IVIG, compared with controls [11]. Here, we report the results of the first multicenter, placebo-controlled trial to evaluate the safety and efficacy of IVIG as adjunctive therapy for patients with STSS.

## SUBJECTS, MATERIALS, AND METHODS

***Study protocol.*** A multicenter, randomized, double-blind, placebo-controlled clinical trial of IVIG treatment of patients with STSS, with or without necrotizing fasciitis, was performed. The trial was investigator-driven and involved 17 hospitals in Sweden, Norway, Finland, and The Netherlands. The study was initiated in January 1999 and, on the basis of intent-to-treat analysis, was projected to include a finite number of 120 patients evenly randomized (1:1) to the IVIG and placebo groups. However, as a result of slow patient recruitment, the trial had to be prematurely terminated in May 2001. The study was approved by the regional ethics committee and drug agency authority in each country. Written informed consent was obtained from all subjects or their legal guardians. The clinical coordinating center (Huddinge University Hospital, Stockholm, Sweden) was available 24 h per day throughout the study to answer investigators' questions regarding patients' eligibility and safety issues and to handle the reporting of serious adverse events. The study was performed in accordance with the Declaration of Helsinki.

***Selection criteria.*** Adult patients (age, $\geq$18 years) who fulfilled the clinical criteria for STSS, including hypotension and multiorgan failure, as described in the consensus definition that was proposed by the Working Group on Severe Streptococcal Infections [1], were eligible for enrollment. Patients could be enrolled before results from bacteriological cultures were obtained if they had clinical symptoms of STSS and if a streptococcal infection was suspected. Suspicion of a streptococcal infection included tissue involvement; suspected tissue involvement as indicated by severe local pain; recent history of streptococcal infections in the family or symptoms of tonsillitis, pharyngitis, or scarlet fever–like rash; or local wound with inflammation. To facilitate identification of GAS cases, throat swabs and aspirates from patients with skin or soft-tissue infections were screened using rapid antigen tests (Strept-A Plus OBC II; Abbott Laboratories, Diagnostics Division). Exclusion criteria included known hypersensitivity to IVIG or underlying diseases that were expected to cause death within 3 months.

***Treatment assignments.*** Patients were randomly assigned in a 1:1 manner to receive either IVIG (Endobulin S/D; Baxter) or an equal volume of 1% albumin (5% albumin [Baxter], diluted in 0.9% normal saline). IVIG was provided intravenously for 3 consecutive days at 1 g/kg of body weight on day 1 and 0.5 g/kg on days 2 and 3. Patients received clindamycin (600 mg iv t.i.d.) in combination with intravenous benzylpenicillin corresponding to a maximum dosage of 12 g per day, for a minimum of 3 days after inclusion. In the case of penicillin intolerance, cefuroxime was provided in dosages of 1.5 g 3 times per day. The total period of antibiotic treatment was standardized to at least 14 days. Plasmapheresis was not allowed to be performed for enrolled patients because it would likely interfere with the study therapy.

***Data collection.*** The study included 5 phases: screening, baseline (maximum of 4 h), treatment (days 1–14), follow-up (days 14–28), and long-term follow-up (days 28–180). At baseline, demographic and clinical information was obtained, including a Simplified Acute Physiology Score II to determine the severity of illness [12]. Organ failures were defined by means of the criteria outlined in the consensus definition for STSS proposed by The Working Group on Severe Streptococcal Infections [13]. Information concerning the infection, including progression of tissue infection (i.e., time to cessation of skin involvement), number of tissue lesions, microbiological data, and antibiotic use, was gathered during all study phases. Other clinical and laboratory data were followed as long as the patient required intensive care. Severity of organ dysfunction was determined on days 1–3 during the stay in the intensive care unit by the Sequential Organ Failure Assessment (SOFA) score [13]. Blood samples for in vitro studies were obtained before infusion of study drug on days 1–3, as well as postinfusion day 3 and on days 28 and 180. Bacterial isolates were recovered from all patients with positive blood culture results. In cases in which blood isolates were not available, isolates recovered from throat or skin/tissue aspirates were obtained.

***Study variables.*** The primary objective was to determine whether IVIG therapy resulted in a decrease in mortality during the first 28 days, compared with placebo. The secondary objective was to evaluate whether there were differences between the 2 groups in time to resolution of shock, time to no further progression of the tissue infection, and survival on day 180, or differences in mental, renal, respiratory, or cardiovascular problems at day 180.

The safety of the study drugs was assessed by an independent data and safety monitoring committee that reviewed nonserious adverse events, serious adverse events, disease-related events, indices of organ dysfunction (hepatic, renal, pulmonary, ce-

**Table 1. Inclusion characteristics of the patients provided high-dose intravenous polyspecific IgG or placebo.**

| Characteristic | IVIG group ($n$ = 10) | Placebo group ($n$ = 11) |
|---|---|---|
| Age, years | | |
| Mean | 51.3 | 52.6 |
| Median (range) | 54 (28–72) | 52 (35–83) |
| Male sex | 4 (40) | 6 (55) |
| SAPS II score[a] | | |
| Mean | 53 | 51 |
| Median (range) | 52 (28–78) | 54 (28–98) |
| SOFA score[a] | | |
| Mean | 11.0 | 11.0 |
| Median (range) | 10.7 (6–16) | 11.5 (6–19) |
| Definitive STSS | 8 (80) | 10 (91) |
| Other TSS | 2 (20)[b] | 1 (9)[c] |
| Deep-tissue infection | 6 (60) | 7 (64) |
| No. of lesions | | |
| Mean | 1.7 | 1.8 |
| Median (range) | 1 (1–5) | 1 (1–4) |
| Time of hypotension before inclusion, h | | |
| Mean | 33.5 | 23.3 |
| Median (range) | 34.0 (2.5–74.8) | 21.2 (2.9–59.4) |
| Dysfunctional organs or systems | | |
| Renal failure | 7 (70) | 8 (73) |
| Coagulopathy | 6 (60) | 9 (82) |
| Liver failure | 5 (50) | 6 (55) |
| Adult respiratory distress syndrome | 4 (40) | 2 (18) |
| Erythematous rash | 2 (20) | 3 (27) |
| Soft-tissue necrosis | 5 (50) | 5 (45) |
| Total no. of organ failures | | |
| Mean | 3.1 | 3.0 |
| Median | 3.0 | 3.0 |
| IL-6 plasma levels,[a] median ng/mL (range) | 1.4 (0.1–29.1) | 2.3 (0.1–1424) |
| IL-8 plasma levels,[a] median ng/mL (range) | 0.3 (0.1–1.6) | 1.6 (0.1–374) |

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. IVIG, intravenous IgG; SAPS II, Simplified Acute Physiology II; SOFA, Sepsis-related Organ Failure Assessment; STSS, streptococcal toxic shock syndrome; TSS, toxic shock syndrome.

[a] Values obtained during screening phase.

[b] One patient with mixed infection caused by *Staphylococcus aureus* and group B streptococcus, and one patient with mixed anaerobic infection in which gram-positive cocci were identified.

[c] Infection caused by *Pseudomonas aeruginosa.*

rebral, cardiac, metabolic, and hematologic), and deaths due to all causes.

***Strain characterization.*** Clinical isolates were identified as GAS by conventional methods. T-typing of the GAS isolates was performed at the Swedish Institute for Infectious Disease Control (Solna, Sweden), in accordance with standard agglutination procedures. DNA from the streptococcal isolates was prepared as described elsewhere [14]. PCR amplification that used primer pairs specific for each superantigen gene was performed to detect genes encoding the streptococcal superantigen *(ssa)* and the pyrogenic exotoxins *spe*A ,*spe*B, *spe*F, *spe*G, *spe*H, and *sme*Z/2, as previously described [15].

***Preparation of bacterial culture supernatant.*** Isolates were cultured overnight in 15 mL Todd-Hewitt broth (Difco) supplemented with 1.5% yeast extract (Difco) at 37°C. Culture supernatants were separated from the bacteria by centrifugation, and proteins in the culture supernatants were ethanol precipitated overnight, as previously described in detail [16].

**Table 2. Characterization of group A streptococcal isolates' serotype and genotype distribution.**

| Strain characteristics | No. (%) of strains | |
|---|---|---|
| | IVIG group[a] ($n$ = 7) | Placebo group[b] ($n$ = 10) |
| T serotype | | |
| T1 | 1 (14) | 7 (70) |
| T12 | 3 (43) | 0 (0) |
| NT | 2 (29) | 1 (10) |
| T3/T3.B3264 | 0 (0) | 2 (20) |
| T4 | 1 (14) | 0 (0) |
| *spe* genotype[c] | | |
| *spe*A | 3 (43) | 8 (80) |
| *spe*H | 5 (71) | 4 (40) |
| *ssa* | 3 (43) | 1 (10) |
| *spe*A and *spe*H | 2 (29) | 3 (30) |
| *spe*H and *ssa* | 3 (43) | 1 (10) |
| *spe*A, *spe*H, and *ssa* | 2 (29) | 1 (10) |
| Mean no. of superantigen genes | 5.6 | 5.2 |

**NOTE.** IVIG, intravenous IgG; NT, not typable; *spe,* streptococcal pyrogenic exotoxin; *ssa,* streptococcal superantigen.

[a] Seven strains available; 2 patients were infected with other bacteria, and 1 had only a positive group A streptococcal rapid antigen test result.

[b] Ten group A streptococcal strains available; 1 placebo patient had infection caused by a non–group A streptococcal isolate.

[c] All strains harbored the *spe*B, *spe*F, *spe*G (except 1), and *sme*Z (except 1) genes.

The supernatants were tested by dose-response experiments to determine the optimal concentration to be used for the neutralizing assay, and a 1:100 dilution was found to be optimal for all isolates. Cell-free culture supernatants were stored at −20°C until used.

***Cytokine assessment at the single-cell level.*** PBMCs were isolated from patient blood by Ficoll-Hypaque gradient centrifugation, transferred to adhesion glass slides (Erie Scientific), and fixed with 2% formaldehyde (Fisher Scientific). The cells were stained for specific cell markers and cytokines via immunohistochemistry, as previously described in detail [17]. In brief, the cells were permeabilized with balanced salt solution–saponin to allow intracellular staining. The following monoclonal antibodies were used: antihuman TNF-$\alpha$ (cocktail of *Mab*I and *Mab*II, murine IgG1; Pharmingen), antihuman IFN-$\gamma$ (cocktail of 1-DIK and 7-B6-1, murine IgG1; MabTech; both used at a concentration of 2 $\mu$g/mL). Antihuman CD3 (Leu 4, murine IgG1, Becton Dickinson; used at a 1:10 dilution), and antihuman CD68 (EMB II, murine IgG1; Dako; used at a 1:200 dilution). A biotinylated goat antimouse IgG1 (Caltag) was used as secondary antibody. The immunohistochemical staining was achieved using avidin-peroxidase reagent (Vectastain Elite-ABC; Vector Laboratories) in combination with the substrate diaminobenzidine (Vector Laboratories). Duplicate fields were stained for the cytokines and single fields for the cell markers. Frequencies of cytokine-producing cells and specific cell types were assessed by direct microscopy, and 500–700 cells were counted per field.

***Assessment of plasma cytokine levels.*** IL-6 and IL-8 concentrations in patient plasma were determined by multiplex cytokine analyses using Fluorokine MAP kits (R&D Systems) and the Luminex 100 instrument (Luminex), in accordance with the manufacturers' instructions.

***Neutralization assay.*** PBMCs were isolated from a healthy donor by Ficoll-Hypaque gradient centrifugation. The PBMCs ($1 \times 10^6$ cells/mL) were cultured in RPMI 1640 medium supplemented with 25 mmol/L HEPES, 4 mmol/L L-glutamine, and 100 U/mL penicillin-streptomycin. The cells were stimu-

**Table 3. Primary and secondary end points of a study assessing the efficacy of administration of high-dose intravenous polyspecific IgG.**

| End point | All included patients | | Patients with GAS only | |
|---|---|---|---|---|
| | IVIG group ($n$ = 10) | Placebo group ($n$ = 11) | IVIG group ($n$ = 8) | Placebo group ($n$ = 10) |
| Primary: mortality day 28, no. (%) of patients | 1 (10) | 4 (36) | 1 (12.5) | 3 (30) |
| Secondary | | | | |
| Time to resolution of shock,[a] h | | | | |
| Mean | 88 | 122 | 100 | 122 |
| Median (range) | 96 (2–159) | 108 (47–294) | 108 (2–159) | 108 (47–294) |
| Time to no further progression of NF/cellulitis, h | | | | |
| Mean | 68[b] | 36[c] | 69[c] | 36[c] |
| Median (range) | 20 (2–168)[b] | 24 (19–72)[c] | 20 (2–168)[c] | 24 (19–72)[c] |
| Mortality day 180, no. (%) of patients | 2 (20) | 4 (36) | 1 (12.5) | 3 (30) |

**NOTE.** GAS, group A streptococci; IVIG, intravenous IgG; NF, necrotizing fasciitis.

[a] In the survivors.

[b] Seven patients.

[c] Five patients.

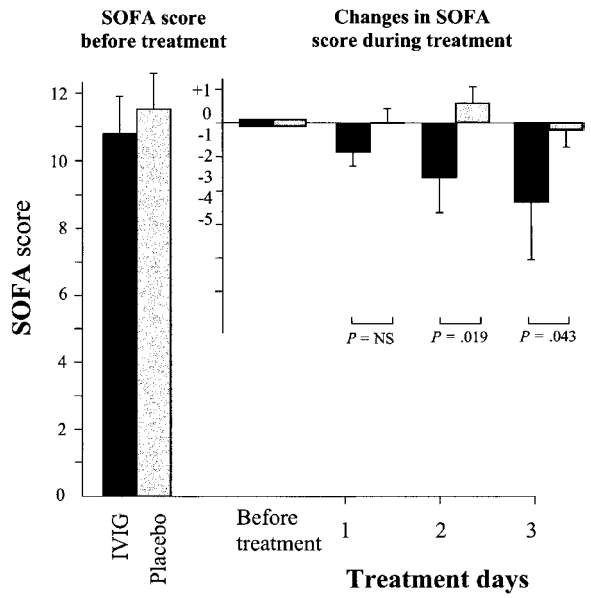


**Figure 1.** Initial Sepsis-related Organ Failure Assessment (SOFA) scores and changes (mean ± SE) during treatment in polyspecific intravenous IgG (IVIG)– and placebo-treated patients. Differences were analyzed by the Mann-Whitney *U* test, and *P* values are indicated.

lated with the cell-free bacterial culture supernatants (diluted 1:100), in the presence of 2.5% heat-inactivated plasma, supplemented with 2.5% heat-inactivated fetal calf serum, as previously described [16]. Patients' plasma samples were tested for inhibitory activity against their own bacterial isolate. After 72 h, the cells were pulsed for 6 h with 1 $\mu$Ci per well of [$^3$H]-thymidine (specific activity, 6.7 Ci/mmol; ICN Biomedicals). Phytohemagglutinin-L (Sigma) was used as a positive control at a concentration of 1 $\mu$g/mL. All samples were assayed in triplicate. The 2 IVIG batches used in the trial were tested for neutralizing activity against culture supernatants from all clinical isolates essentially as described above, but cells were stimulated in the presence of 5% fetal calf serum.

***Statistical analysis.*** The binary data on mortality were analyzed by Fisher's exact test. For all other comparisons, the Wilcoxon-Mann-Whitney *U* test was used. This test was also used for analyses of cytokines and differences in the neutralizing capacity of bacterial culture supernatants by patient plasma. The *P* values are based on 2-sided tests.

## RESULTS

The trial was terminated prematurely as a result of slow patient recruitment. The main reason for the slow patient recruitment seemed to be a low incidence of STSS in the participating countries during the trial period. Only 21 patients, 10 of whom received IVIG and 11 of whom received placebo, were included (table 1). All surviving enrolled patients were observed for the entire study period. GAS strains were isolated from 17 of the patients. For 1 patient, the strain could not be cultured because of previous antibiotic therapy, but GAS was implicated as the causative agent on the basis of a positive result of a rapid antigen test performed on aspirate samples from the affected skin area. The majority (94%) of the GAS isolates were obtained from sterile sites—that is, blood cultures (53%) or cultures of tissue samples (41%). The nonsterile isolate was obtained from a throat swab. In 3 patients with suspected STSS, non-GAS strains were isolated from blood cultures, including *Pseudomonas aeruginosa,* group B streptococcus in combination with *Staphylococcus aureus,* and a mixed anaerobic infection in which gram-positive cocci had been observed by Gram stain and direct microscopy.

At the time of inclusion in the trial, clinical and demographic characteristics were similar in the IVIG and placebo groups (table 1). Levels of IL-6 and IL-8 were found to be somewhat higher in the plasma samples obtained from the placebo group before study drug infusion day 1 (table 1). There was an overrepresentation of T1 and T3 isolates, and there was a higher prevalence of the *spe*A gene among strains isolated from the

**Table 4. Clinical characteristics of patients who died who received high-dose intravenous polyspecific IgG (IVIG) or placebo.**

| Treatment | Age, years | Sex | Days to death | Deep-tissue infection | Duration of hypotension before inclusion, h | Total no. of organ failures at inclusion | Clinical isolate | Superantigen genotype profile |
|---|---|---|---|---|---|---|---|---|
| IVIG | 72 | F | 163 | Yes | 11 | 3 | GBS + *S. aureus* | NA |
| IVIG | 28 | F | 2 | No | 38 | 4 | GAS T4[a] | *spe*A$^-$, G$^-$, H$^-$, *ssa*$^+$ |
| Placebo | 83 | F | 1 | No | 5 | 2 | *P. aeruginosa* | NA |
| Placebo | 64 | M | 8 | No | 40 | 3 | GAS T1[a] | *spe*A$^+$, G$^+$, H$^-$, *ssa*$^-$ |
| Placebo | 60 | F | 7 | Yes | 26 | 4 | GAS T3[a] | *spe*A$^+$, G$^+$, H$^-$, *ssa*$^+$ |
| Placebo | 67 | F | 15 | Yes | 34 | 2 | GAS NT[a] | *spe*A$^-$, G$^+$, H$^+$, *ssa*$^-$ |

**NOTE.** GAS, group A streptococci; GBS, group B streptococci; NA, not applicable; NT, not typable; *P. aeruginosa, Pseudomonas aeruginosa; S. aureus, Staphylococcus aureus; spe,* streptococcal pyrogenic exotoxin; *ssa,* streptococcal superantigen.

[a] Indicates serotype of GAS strain.

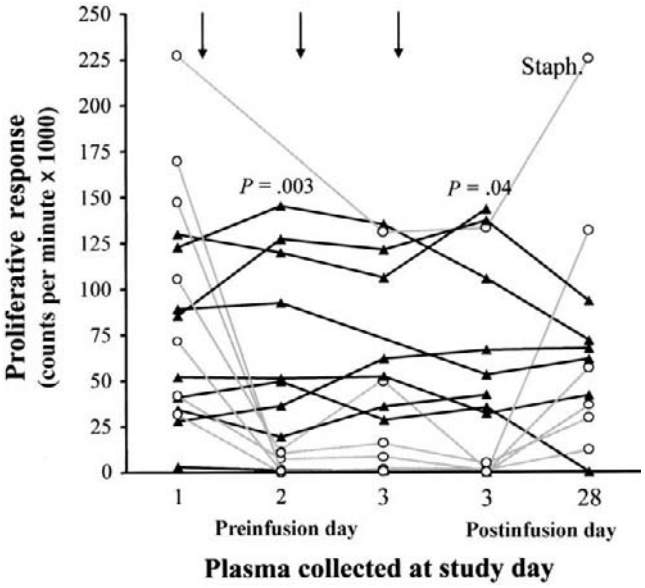


**Figure 2.** Neutralization of proliferative activity of bacterial culture supernatants by patient plasma. Plasma from patients treated with polyspecific intravenous IgG (IVIG; *open circles*) or placebo *(filled triangles)* were tested for neutralizing activity against culture supernatants prepared from the patients' own isolates, as detailed in Subjects, Materials, and Methods. One patient was infected with *Staphylococcus aureus* (Staph.), as indicated. Study drug was administered on days 1, 2, and 3, as indicated by arrows. Plasma samples obtained preinfusion on days 1, 2 and 3, on postinfusion day 3, and at follow-up on day 28 were analyzed. Differences were analyzed by the Mann-Whitney *U* test, and *P* values are indicated.

placebo group, compared with the IVIG group (table 2). However, the majority of strains harbored genes encoding for 5–7 different superantigens, and there was no difference between the groups with regard to the total number of superantigen genes.

The primary end point was mortality over 28 days. The mortality rate was 3.6-fold higher in the placebo group than in the IVIG group, but statistical significance was not reached ($P = .3$), presumably because of the small sample size (table 3). No significant differences could be seen for the secondary end points. However, the reduction in the SOFA score was significantly higher in the IVIG group than in the placebo group on days 2 and 3 ($P = .02$ and .04, respectively; figure 1). Similar results for primary and secondary end points were obtained when only data from patients with proven STSS were analyzed (table 3). A change in the SOFA score was also observed when only patients with proven STSS were included in the analysis, but the change in the SOFA score was significant only on day 2, the mean values being −1.1, −1.6, and −2.3 on treatment days 1, 2, and 3, respectively.

Clinical and strain characteristics of the fatal cases are shown in table 4. Patients who died did not differ from survivors with respect to duration of hypotension before inclusion in the study, number of organ failures, or prevalence of deep-tissue infection. The mean age of patients who died was 62 years, compared with 48 years for survivors. The serotypes of GAS isolates varied among the patients who died and included T1, T3, T4, and a nontypeable strain. Also, the superantigen genotype profile of the strains varied. In 2 patients who died, bacteria other than GAS caused the episodes of toxic shock.

Cytokine assessment at the single-cell level revealed no differences between the groups or within treatment groups over time with respect to frequencies of TNF-α– and IFN-γ–producing cells, or CD3- and CD68-positive cells (data not shown). Analyses of plasma cytokine levels showed a gradual decrease in IL-6 and IL-8 levels over time in all patients; this did not differ between the IVIG and placebo groups.

The results obtained from the neutralization assays are summarized in figure 2. In the placebo group, all plasma samples obtained before or after therapy lacked neutralizing activity against the tested GAS supernatants. In the IVIG group, low neutralizing activity was found in plasma before therapy. All patients except one attained neutralizing activity in their plasma after administration of IVIG. The IVIG patient who did not attain neutralizing activity against her isolate was the patient who had a mixed infection caused by a group B streptococcal strain and a *S. aureus* strain. Since the group B streptococcal isolate did not induce a proliferative response when tested in vitro, the plasma neutralizing assay was performed by using the *S. aureus* isolate. Significant differences between the treatment groups were found at day 2 and after infusion of study drug on day 3; the IVIG group had significant higher neutralizing activity ($P = .003$ and .04, respectively). In vitro experiments showed that all GAS strains were inhibited 87%–100% by the 2 IVIG batches used in the study, whereas the *S. aureus* strain was only inhibited to 65% (data not shown).

Reported were 6 severe adverse events (i.e., deaths) and 12 adverse events or disease-related events, including arterial fibrillation [2], diarrhea, prostatitis, cerebral embolus or epilepsy, lung bleeding, candida septicemia, hypotension, adult respiratory distress syndrome/pneumonia, soft-tissue necrosis, fever not responsive to antimicrobial treatment, and epileptic convulsion. None of the events were reported to be related to the study drug.

## DISCUSSION

Several case reports have described the use of IVIG therapy in patients with severe invasive GAS infections, including STSS, necrotizing fasciitis, and necrotizing myositis [18–26]. Support for a clinical efficacy of IVIG in STSS was provided by an observational cohort study of IVIG therapy conducted in Canada, which reported a significantly reduced mortality rate

among IVIG-treated patients than among control subjects [11]. However, confounding factors in this study that potentially affected mortality data included study of historical controls and increased use of clindamycin among IVIG-treated patients.

To provide evidence for a clinical efficacy of IVIG in STSS, we conducted a multicenter placebo-controlled trial designed to evaluate the efficacy and safety of high-dose IVIG in this disease setting. However, as a result of a low incidence of STSS in the participating countries during the study period, patient recruitment was slow, and the trial was prematurely terminated after enrollment of 21 patients. The IVIG and placebo groups were well matched with respect to clinical and demographic characteristics, but there were differences in strain characteristics between the groups. Serotypes T1 and T3 were overrepresented among isolates from the placebo group, and the *spe*A gene, which is commonly found in these serotypes, was consequently more prevalent in strains isolated from the placebo group.

However, and most importantly, all GAS strains harbored genes encoding for several different superantigens, and there was no difference between the groups in the total number of superantigen genes. Although the T1M1 and T3 serotypes have been the most common serotypes found in the recent outbreaks of severe streptococcal infections and, therefore, are believed to be more invasive, other serotypes are known to cause severe and even fatal infections [4, 27–35]. This point is underscored in the current study, in which isolates recovered from the patients who died included serotypes of T1, T3, and T4, as well as a nontypeable strain. Furthermore, the superantigen gene profile differed in these isolates, with 2 strains lacking the *spe*A gene. Therefore, we find it unlikely that the overrepresentation of T1 and T3 strains in the placebo group would significantly affect the outcome of the study.

Analyses of the primary end point revealed a trend toward a reduced mortality rate in IVIG-treated patients as compared with those receiving placebo (10% vs. 36%). These results were supported by significantly better improvement of organ dysfunction during treatment days 1–3 in the IVIG group, as assessed by the SOFA score, which has been considered especially suitable for sequential analyses [13].

We also performed in vitro studies to detect any differences between the IVIG and placebo groups with respect to superantigen-neutralizing activity and cytokine levels. No difference between the groups was noted in the cytokine levels before and after therapy. Both groups showed an equal decrease in the levels of serum IL-6 and IL-8 on day 2. A significant increase in superantigen-neutralizing activity after administration of IVIG could be seen, whereas plasma neutralizing activity remained low in the placebo group. This is in agreement with previous reports that IVIG confers neutralizing antisuperantigen antibodies to the patients [11, 16, 36].

Earlier reports have indicated that there may be a rapid consumption of the relevant antibodies in patients with STSS and that multiple doses of IVIG may be required to maintain an adequate level of neutralization of superantigens [11, 16, 36]. In this study, the patients received 3 infusions of IVIG with the highest dosage (1 g/kg body weight) provided at day 1, followed by 0.5 g/kg on days 2 and 3. Comparison of plasma samples obtained after the first dose of IVIG (sample obtained before treatment day 2) and before the third dose on day 3 also indicated a rapid consumption of antibodies: 83% of the samples showed a reduction in neutralizing activity from day 2 to day 3.

The results of this study, even though they did not reach significant differences in the primary end point, provide further support for the use of IVIG as adjunctive therapy in patients with STSS. Although it would be desirable to have a larger controlled trial to provide statistical support for a clinical efficacy, the low incidence of STSS is a major obstacle that caused a premature termination of the present study and is likely to discourage the conduct of further trials. Considering this as well as the high mortality rate and the clinical and functional data published to date, it seems reasonable to recommend IVIG as adjunctive therapy for the treatment of STSS until further data are available.

## StreptIg STUDY GROUP

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***Streptococcal serotyping.*** Birgitta Henriques Normark.

***Data and safety committee.*** Johan Giesecke, Göran Hermerén, and Jørgen Hilden.

***Scientific advisor.*** Don E. Low.

## Acknowledgments

The gift of group A streptococcal rapid antigen tests by Abbott Scandinavia AB is acknowledged. We also acknowledge the

assistance of the pharmacies and health care workers at the participating centers.

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