ORIGINAL ARTICLE

Improving outcomes of severe infections by multidrug-resistant pathogens with polyclonal IgM-enriched immunoglobulins

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Abstract

The emergence of infections by multidrug-resistant (MDR) Gram-negative bacteria, which is accompanied by considerable mortality due to inappropriate therapy, led to the investigation of whether adjunctive treatment with one polyclonal IgM-enriched immunoglobulin preparation (IgGAM) would improve outcomes. One hundred patients in Greece with microbiologically confirmed severe infections by MDR Gram-negative bacteria acquired after admission to the Intensive Care Unit and treated with IgGAM were retrospectively analysed from a large prospective multicentre cohort. A similar number of patient comparators well-matched for stage of sepsis, source of infection, appropriateness of antimicrobials and co-morbidities coming from the same cohort were selected. All-cause 28-day mortality was the primary end point; mortality by extensively drug-resistant (XDR) pathogens and time to breakthrough bacteraemia were the secondary end points. Fifty-eight of the comparators and 39 of the IgGAM-treated cases died by day 28 (p 0.011). The OR for death under IgGAM treatment was 0.46 (95% CI 0.26–0.85). Stepwise regression analysis revealed that IgGAM was associated with favourable outcome whereas acute coagulopathy, cardiovascular failure, chronic obstructive pulmonary disease and chronic renal disease were associated with unfavourable outcome. Thirty-nine of 62 comparators (62.9%) were infected by XDR Gram-negative bacteria and died by day 28 compared with 25 of 65 cases treated with IgGAM (38.5%) (p 0.008). Median times to breakthrough bacteraemia were 4 days and 10 days, respectively (p <0.0001). Results favour the use of IgGAM as an adjunct to antimicrobial treatment for the management of septic shock caused by MDR Gram-negative bacteria. A prospective randomized trial is warranted.

Keywords: Antimicrobial resistance, bacteraemia, IgM immunoglobulin, mortality, septic shock
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Introduction

In many parts of the world, species of Gram-negative bacteria that are multidrug-resistant (MDR) to commonly prescribed antimicrobials predominate as pathogens of severe sepsis [1,2]. These MDR species emerge after exposure of patients to

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antimicrobials and to healthcare settings. In Greece, MDR species of *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are the commonest pathogens of severe infections acquired after admission to Intensive Care Units (ICU); resistance to carbapenems exceeds 65% [3].

High resistance rates generate major problems of inappropriate antimicrobial therapy with poor outcomes, highlighting an unmet need for treatment. These severe infections are also characterized by impairment of the innate immune defence for effective phagocytosis of bacteria [4]. Enhancement of the opsonization by IgM may be a promising strategy for the containment of MDR Gram-negative bacteria. IgM forms pentamers that opsonize invading pathogens and facilitate phagocytosis. This was shown by the promising results of one prospective open-label single-arm study in a limited number of patients with ventilator-associated pneumonia (VAP) caused by isolates of *P. aeruginosa* serotype O11 [5]. As a next step, it is expected that the use of existing immunoglobulin preparations enriched with IgM will be more effective than the use of IgM antibodies directed against specific sites due to the polyvalence of polyclonal preparations. The only available polyclonal preparation enriched with IgM immunoglobulins is Pentaglobin® (Biotest AG, Dreieich, Germany), which contains 76% IgG, 12% IgA and 12% IgM (IgGAM). IgGAM induces *in vitro* killing of MDR clinical isolates of *K. pneumoniae* through enhancement of phagocytosis [6].

The Hellenic Sepsis Study Group (HSSG, www.sepsis.gr) is collecting a prospective cohort of clinical data from patients with severe infections from Greek hospitals. We conducted a retrospective analysis of the outcome of severe infections due to MDR Gram-negative pathogens developing after ICU admission for which treatment with IgGAM was administered; results were compared with those of untreated matched comparators from the same departments at the same time period.

Patients and methods

Study design

A prospective registry protocol has been running since July 2006 at 63 study sites of the HSSG in Greece after approval by the Ethics Committees of the participating hospitals. Approval was granted for the analysis of the use of antimicrobial and adjunctive therapies administered to enrolled patients at the discretion of the attending physicians. Patients are enrolled after written informed consent to use their clinical data for this analysis; consent is provided by the patients themselves or by first-degree relatives for patients unable to consent. Sepsis stages and infections are defined by internationally accepted definitions [7,8]. Clinical data are prospectively collected for 28 days and registered in a central database. No patients have been treated with recombinant human activated protein C. A search of the database conducted in September 2015 indicated 232 patients with ICU-acquired sepsis who were treated with IgGAM. It was then decided by a panel of experts (AA, EA, CR, EJGB, GV, KM, NK and VK) to design a protocol in which patients treated with IgGAM and well-matched comparators would be further analysed. Inclusion criteria were: (a) severe sepsis or septic shock; (b) primary or secondary bacteraemia or VAP by one MDR Gram-negative pathogen isolated either from the blood or from tracheobronchial secretions or from bronchoalveolar lavage; isolates from quantitative cultures of tracheobronchial secretions or from bronchoalveolar lavage at counts $>10^5$ CFU/mL or 10^4 CFU/mL, respectively, were eligible; and (c) start of IgGAM <24 h from the development of the first signs of infection. An isolate resistant to antimicrobials of at least three different chemical classes was considered MDR [9]. Isolates susceptible to only one antimicrobial were considered extensively drug-resistant (XDR) [9]. Exclusion criteria were: (a) infection by the human immunodeficiency virus; (b) neutropenia defined as <1000 neutrophils/mm^3; (c) chronic intake of corticosteroids defined as the intake of >0.4 mg/kg of equivalent prednisone daily for >15 consecutive days; (d) any primary immunodeficiency; (e) isolation of coagulase-negative *Staphylococcus* spp. and of skin commensals; (f) catheter-related infections; (g) more than one isolate from tracheobronchial secretions or from bronchoalveolar lavage; and (h) end of life (do not resuscitate) decision.

The following information was analysed per patient: demographics, co-morbidities, Charlson's co-morbidity index (CCI) [10], type of infection, failing organs, Acute Physiology and Chronic Health Evaluation (APACHE) II score, Sequential Organ Failure Assessment (SOFA) score, daily dose and total duration of IgGAM treatment, microbiology and antibiogram of the isolated microorganism, type and dose of administered antimicrobial(s), appropriateness of the administered antimicrobial(s), white blood cell count, daily blood cultures and 28-day outcome. Appropriateness of the administered antimicrobial(s) was defined as the administration of at least one antimicrobial active against the isolated pathogen according to the antibiogram. Organ failures were defined according to international definitions [7]. Breakthrough bacteraemia was considered as the advent of a new episode of bloodstream infection developing in a patient with sterile blood cultures for at least 72 h.

The comparators were selected among patients of the HSSG database to be of the same number as the IgGAM group using a stepwise matching process. Matching criteria were predefined by the same expert panel and required the same inclusion and exclusion criteria as the IgGAM group. The following steps applied

for selection: (a) patients with ICU-acquired sepsis from the same study sites and the same year; (b) severe sepsis/septic shock; (c) appropriateness of empirical antimicrobial treatment; (d) source of infection by MDR Gram-negative bacteria; and (e) CCI.

Study end points

The study end points and the statistical methods to be used were predefined. The primary end point was all-cause 28-day mortality. The secondary end points were: (a) the impact of IgGAM in the 28-day outcome of infections by XDR bacteria; and (b) the impact of IgGAM on the time until breakthrough bacteraemia. *Post hoc* analysis was also performed for the impact of IgGAM on patient subgroups.

Statistical analysis

The matching procedure was performed using the case–control matching command found in IBM SPSS Statistics version 22. This command requires the Python Essentials. Comparisons of baseline characteristics were performed with the independent samples *t*-test for continuous variables and with the chi-square test for frequency distributions. The primary outcome was compared between the two groups with the Fisher exact test. The OR were also reported with their 95% CI. The 28-day survival distributions of the two groups were compared with Kaplan–Meier survival analysis using the log-rank test. The same analysis was applied with regards to the secondary end points.

Univariate analyses for qualitative variables between survivors and non-survivors were carried out by the Fischer exact test. Then a stepwise logistic regression model was applied with outcome on day 28 as the dependent variable and all the variables found to have a significant effect on the outcome at the univariate analysis as the independent predictors. Finally, based on the results of the stepwise logistic regression, the primary outcome was once again regressed across the two groups (comparators versus IgGAM) in the absence and presence of the conditions found to have a significant effect on 28-day mortality. The aim was to find in which conditions the administration of IgGAM was more beneficial.

All the above analyses were performed with the IBM SPSS Statistics version 22. The statistical significance level was set to 0.05.

Results

Groups of comparisons and matching process

From the 222 patients treated with IgGAM, 100 met all the inclusion criteria and none of the exclusion criteria (Fig. 1).

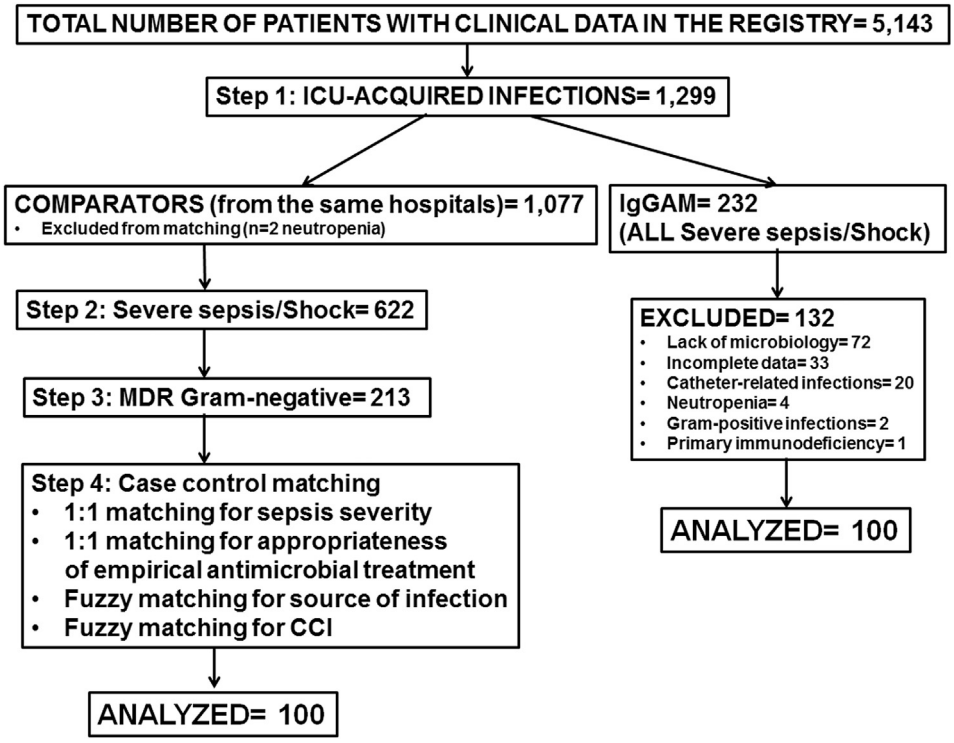

FIG. 1. Case–control matching process. Abbreviations: CCI, Charlson's Co-morbidity Index; IgGAM, IgM-enriched polyclonal immunoglobulin preparation; MDR, multidrug-resistant.

Following the stepwise selection and matching procedure shown in Fig. 1, 100 comparators meeting all the same inclusion criteria and none of the exclusion criteria were selected.

The mean daily dose of IgGAM was 30 g and it was administered as a 5- to 6-h continuous daily infusion for 5 days. Baseline characteristics of each group are shown in Table 1. Cases treated with IgGAM had a significantly greater baseline disease severity, as shown by the significantly greater SOFA score and the greater proportion of multiple organ dysfunction syndrome. The frequency of acute coagulopathy was greater in the IgGAM group at baseline, i.e. before the start of treatment with the IgGAM preparation. Empirically prescribed antimicrobial treatment on day 1 did not differ between groups (see Supplementary material, Table S1) and the same applied for the resistance rates of the isolated pathogens (see Supplementary material, Fig. S1).

Primary end point: all-cause 28-day mortality

The all-cause 28-day mortality among the comparators was 58/100 whereas in the IgGAM group it was 39/100 (p 0.011). For death under IgGAM the OR was 0.46 (95% CI 0.26–0.81). The statistically significant differences pertain with regards to the 28-day survival curves in the IgGAM and the comparator group (Fig. 2).

TABLE 1. Comparative characteristics of cases treated with an IgM-enriched polyclonal immunoglobulin preparation (IgGAM) and of matched comparators

	Comparators (n = 100)	IgGAM (n = 100)	p
Gender, male/female	66/34	70/30	0.544
Age (years), mean ± SD	54.2 ± 18.4	51.9 ± 18.6	0.399
Mechanical ventilation	100	100	1.000
Septic shock/severe sepsis	86/14	86/14	1.000
APACHE II score, mean ± SD	20.7 ± 6.6	19.6 ± 6.9	0.229
SOFA score, mean ± SD	8.9 ± 3.3	10.2 ± 3.3	0.013
SOFA subscore for Po_2/Fio_2	2.19 ± 1.25	2.14 ± 1.37	0.788
SOFA subscore for platelets	0.53 ± 1.08	1.36 ± 1.46	<0.0001
SOFA subscore for creatinine	1.33 ± 1.40	0.62 ± 1.07	<0.0001
SOFA subscore for bilirubin	0.68 ± 0.90	1.11 ± 1.03	0.003
SOFA subscore for vasopressors	1.90 ± 1.35	2.09 ± 1.22	0.358
SOFA subscore for Glasgow coma scale	2.17 ± 1.30	2.83 ± 1.51	0.001
White blood cells (/mm^3), mean ± SD	14 509.3 ± 9495.6	13 254.4 ± 9834.9	0.360
Po_2/Fio_2 (mmHg), mean ± SD	218.1 ± 111.6	169.7 ± 133.5	0.012
Appropriateness of empirically administered antimicrobials on day 1, Yes/No	51/49	51/49	1.000
Source of sepsis			0.730
Primary bacteraemia	24	18	
VAP	56	63	
VAP and secondary bacteraemia	18	17	
IAI and secondary bacteraemia	2	2	
Isolated pathogens (n)			0.287
Acinetobacter baumannii	59	55	
Klebsiella pneumoniae	25	30	
Pseudomonas aeruginosa	25	17	
Other	9	10	
Polymicrobial infection (n)	21	13	0.187
Failing organs			
Acute respiratory distress syndrome	57	73	0.026
Acute kidney injury	26	15	0.079
Cardiovascular shock	86	86	1.000
Metabolic acidosis	26	23	0.743
Acute coagulopathy	25	52	<0.0001
Presence of multiple-organ dysfunction	64	84	0.002
Coagulation abnormalities			
Platelets <100 000/mm^3	18	42	<0.0001
International normalized ratio >1.5	31	24	0.265
Reason for ICU admission			
Multiple injuries	22	25	0.739
Acute respiratory failure	21	13	0.187
Community-acquired severe sepsis	17	21	0.471
Stroke	15	9	0.199
Head injury	15	18	0.704
Brain haemorrhage	9	14	0.376
Predisposing conditions			
Type 2 diabetes mellitus	18	18	1.000
Chronic respiratory failure	21	13	0.187
Chronic heart failure	21	16	0.467
Chronic renal disease	10	3	0.082
Malignancy	15	12	0.680
Charlson's co-morbidity index (mean ± SD)	2.86 ± 2.68	2.67 ± 2.43	0.601
Adjunctive therapies			
Vasopressors	86	86	1.000
Low-dose hydrocortisone replacement	44	63	0.007
Tight glucose control	43	32	0.144
Continuous veno-venous haemofiltration	14	22	0.197

Abbreviations: APACHE, acute physiology and chronic health evaluation; IAI, intrabdominal infection; SOFA, sequential organ failure assessment; VAP, ventilator-associated pneumonia.

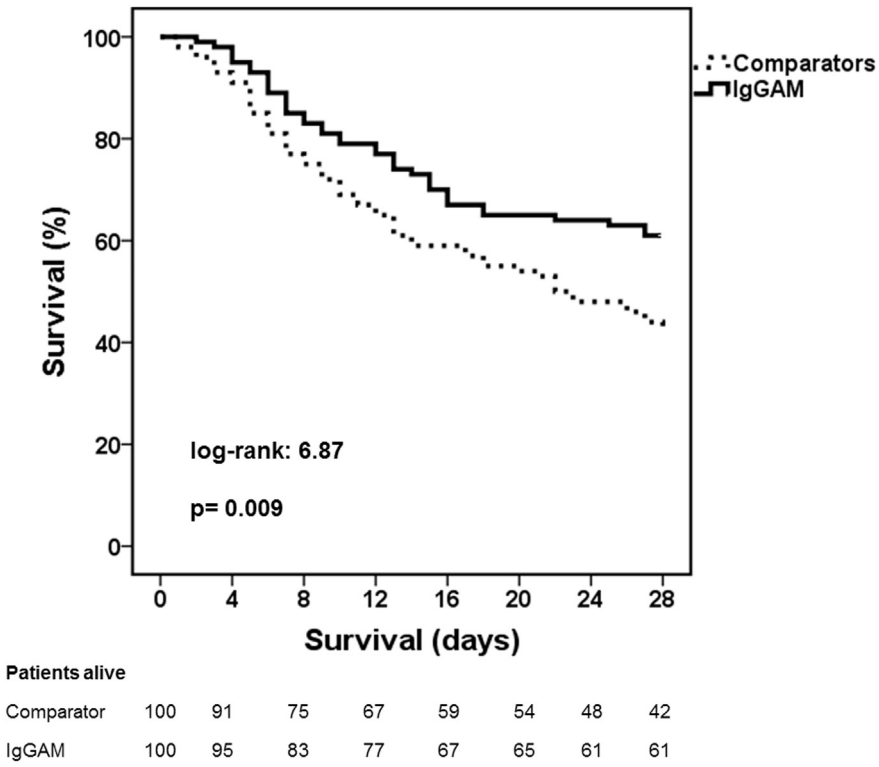

FIG. 2. Survival plots for the patients treated with an IgM-enriched polyclonal immunoglobulin preparation (IgGAM) and the comparator group until day 28; statistical comparison between the two groups is shown.

To identify factors related with unfavourable outcome, univariate analysis was carried out between survivors and non-survivors (Table 2). This analysis showed that treatment with IgGAM, SOFA >10, CCI >2, the presence of acute kidney injury, the presence of acute coagulopathy, the presence of cardiovascular failure, chronic respiratory failure, chronic renal disease, brain haemorrhage and multiple injuries, tight glucose control and continuous veno-venous haemofiltration were related with unfavourable outcome. These factors entered into a stepwise regression analysis model. SOFA >10 and CCI >2 were excluded from this model because they are correlated to the other variables. This model (Table 3) showed that

TABLE 2. Univariate analysis between survivors and non-survivors

Variable[a]	Survivors, (n = 103)	Non-survivors (n = 97)	p	OR	95% CIs
IgGAM treatment	61 (59.2)	39 (40.2)	0.008	0.46	0.26–0.82
SOFA >10[b]	27 (26.2)	44 (45.4)	0.005	2.34	1.29–4.23
Charlson-co-morbidity index >2[b]	36 (35.0)	60 (61.9)	<0.0001	3.02	1.67–5.37
White blood cells (/mm^3), mean ± SD)	12 825.1 ± 8473.4	15 003.9 ± 10 714.3	0.116	1.26	0.94–1.69
ARDS	66 (64.0)	64 (66.0)	0.778	1.09	0.61–1.95
Acute kidney injury	13 (12.6)	28 (28.9)	0.004	2.81	1.36–5.82
Acute coagulopathy	30 (29.1)	47 (48.5)	0.005	2.29	1.28–4.09
Metabolic acidosis	18 (17.5)	31 (32.0)	0.017	2.22	1.14–4.31
Cardiovascular failure	82 (79.6)	90 (92.8)	0.010	3.29	1.33–8.15
Type 2 diabetes mellitus	18 (17.5)	18 (18.6)	0.842	1.08	0.52–2.22
Chronic heart failure	17 (16.5)	20 (20.6)	0.454	1.31	0.64–2.69
Chronic respiratory failure	9 (8.7)	25 (25.8)	0.001	3.68	1.56–8.25
Chronic renal disease	2 (1.9)	11 (11.3)	0.007	6.46	1.39–29.95
Solid tumour malignancy	11 (10.7)	16 (16.5)	0.229	1.65	0.73–3.77
Stroke	10 (9.7)	14 (14.6)	0.291	1.59	0.67–3.77
Brain injury	23 (22.3)	10 (10.4)	0.024	0.40	0.18–0.90
Multiple injuries	34 (33.0)	13 (13.5)	0.001	0.32	0.16–0.65
Brain haemorrhage	15 (14.6)	8 (8.3)	0.170	0.53	0.22–1.32
Low hydrocortisone replacement	50 (48.5)	57 (58.8)	0.148	1.51	0.86–2.64
Tight glucose control	31 (30.2)	44 (45.4)	0.027	1.93	1.08–3.45
Continuous veno-venous haemofiltration	12 (11.7)	24 (24.7)	0.018	2.49	1.17–5.32

Abbreviations: ARDS, acute respiratory distress syndrome; IgGAM, IgM-enriched polyclonal immunoglobulin preparation; SOFA, sequential organ failure assessment.
[a]All values given as n (%) unless otherwise stated.
[b]Refers to the respective median value.

TABLE 3. Results of the stepwise logistic regression model

Predictor	p	OR	95% CI
IgGAM treatment	0.002	0.34	0.17–0.67
Acute coagulopathy	<0.0001	3.53	1.74–7.16
Cardiovascular failure	0.003	4.77	1.68–13.56
Chronic respiratory failure	0.003	3.90	1.61–9.47
Chronic renal disease	0.026	7.18	1.27–40.58

Abbreviations: IgGAM, IgM-enriched polyclonal immunoglobulin preparation.

treatment with IgGAM was an independent factor related with favourable outcome.

The same model showed that acute coagulopathy, cardiovascular failure, chronic respiratory failure and chronic renal disease were independent predictors of unfavourable outcome. Cross-tabulation indicated that treatment with IgGAM was beneficial in patients with cardiovascular failure whereas in the case of acute coagulopathy and chronic respiratory failure, IgGAM did not seem to provide the additional benefit that seemed to provide in the absence of these two conditions (Table 4). Finally, in the case of chronic renal disease, the question of the beneficial effect of IgGAM administration remained unanswered because of the small number of patients.

Secondary end points

Sixty-two patients of the comparator group and 65 patients of the IgGAM group were infected by XDR Gram-negative bacteria; 39 patients (62.9%) and 25 patients (38.5%), respectively, died until 28-day by all-causes (p 0.008). The OR for death by XDR Gram-negative bacteria treated with IgGAM was 0.37 (95% CI 0.18–0.76).

Thirty comparators and 65 patients treated with IgGAM had sterile blood cultures for more than 72 h during their follow up and could be evaluated for the development of breakthrough bacteraemia; 12 patients (40.0%) and 22 (33.8%) patients respectively developed breakthrough bacteraemia (p 0.682). The median time to breakthrough bacteraemia was 4 days for the comparators (range 3–6 days) and 10 days for the IgGAM-treated cases (range 7–12 days) (Fig. 3).

Discussion

The presented analysis comprises a meaningful number of patients with severe infections by MDR Gram-negative bacteria treated with one IgM-enriched immunoglobulin preparation. Results indicate a considerable survival benefit on all-cause 28-day mortality and on delay until one breakthrough bloodstream infection. Analysis indicated that most of the survival benefit was found among patients with cardiovascular failure and for infections by XDR Gram-negative bacteria. This translates to the need for an early start of IgGAM when surveillance cultures suggest a probability for XDR pathogens where the risk for inappropriate initial antimicrobial therapy is high.

The rationale of supplementation with IgM preparations for the management of severe infections is to neutralize bacterial endotoxins and exotoxins and to enhance phagocytosis of evading pathogens. A recent study of the HSSG reported circulating IgM in patients with sepsis, 113 of which were classified as severe sepsis and 78 as septic shock. Considerable reduction of IgM was found in septic shock. When circulating IgM was measured on sequential days after start of vasopressors in septic shock, it was found that the distribution of IgM was lower in non-survivors than in survivors from septic shock [11]. This corroborates very well the reported finding in this analysis where most of the survival benefit was shown in cardiovascular failure; this is the stage of sepsis where deficient distribution of IgM in the host is occurring. Others [12] have shown profound hypoglobulinaemia of not only IgM but also of IgG and IgA immunoglobulins on the first day of septic shock. The studied IgGAM preparation contains IgG immunoglobulins as well, so part of the beneficiary action may be related to the supplementation of missing IgG along with the missing IgM.

TABLE 4. Modulation of the effect of independent predictors of 28-day mortality by treatment with the IgM-enriched polyclonal immunoglobulin preparation (IgGAM)

		Comparators	IgGAM			
Predictor	Condition	Deaths/exposed patients (%)		p	OR	95% CIs
Acute coagulopathy	Absence	41/75 (54.7)	9/48 (18.8)	<0.0001	0.19	0.08–0.45
	Presence	17/25 (68.0)	30/52 (57.7)	0.385	0.64	0.24–1.75
Cardiovascular failure	Absence	4/14 (28.6)	3/14 (21.4)	0.613	0.68	0.12–3.83
	Presence	54/86 (62.8)	36/86 (41.9)	0.004	0.43	0.23–0.79
Chronic respiratory failure	Absence	42/79 (53.2)	30/87 (34.5)	0.015	0.46	0.29–0.87
	Presence	16/21 (76.2)	9/13 (69.2)	0.655	0.7	0.15–3.31
Chronic renal disease	Absence	49/90 (54.4)	37/97 (38.1)	0.025	0.52	0.29–0.92
	Presence	9/10 (90.0)	2/3 (66.7)	0.326	0.22	0.09–5.28

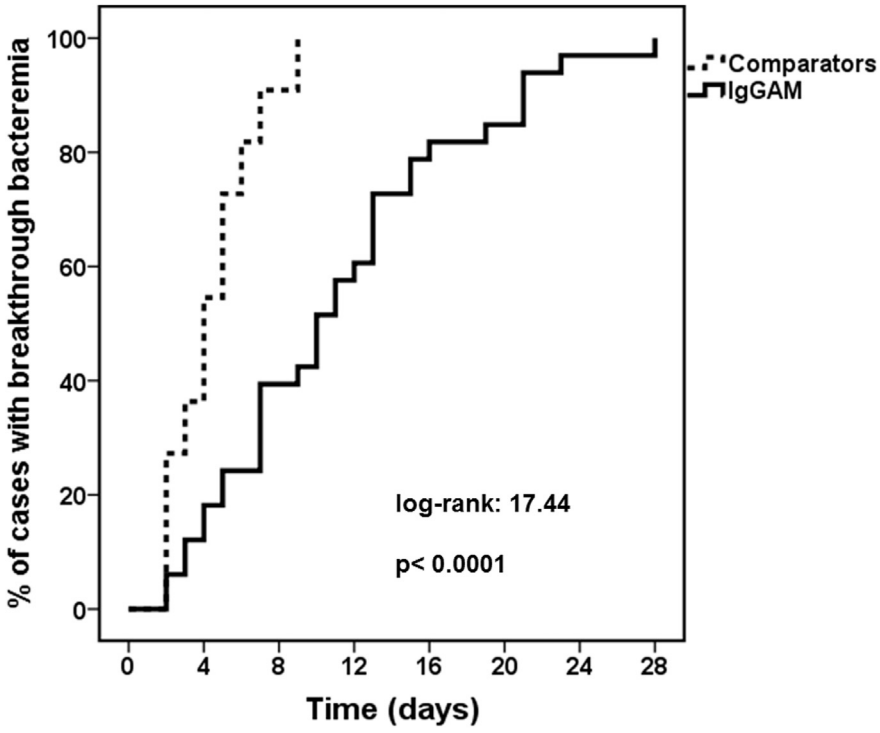

FIG. 3. Time to breakthrough bacteraemia for cases treated with an IgM-enriched polyclonal immunoglobulin preparation (IgGAM) and matched comparators; statistical comparison between the two groups is shown.

Several randomized trials have been published over the last 20 years on the efficacy of immunoglobulin preparations for patients with severe infections. Available trials are characterized by great heterogeneity regarding monoclonality or polyclonality and IgG or IgM content of the administered preparations. Trials have been conducted in both neonates and adults and in most of them the number of enrolled patients is limited. The most recent meta-analysis tried to overcome these sources of bias and reported separately on each situation. Part of the meta-analysis was done with seven trials with a total of 528 adult participants with severe sepsis or septic shock treated either with IgGAM or placebo or no intervention. The OR for all-cause mortality was 0.66 with moderate risk of bias (95% CI 0.51–0.84) [13]. A former meta-analysis comprising ten randomized trials conducted both in neonates and in adults has shown that most of the survival benefit from IgGAM treatment is for patients with septic shock by Gram-negative bacteria [14].

Two main limitations of our study should be recognized: (a) the retrospective nature; and (b) the lack of comparators treated with IgG preparations or some other appropriate comparator like albumin. Despite these limitations, some strong points of the study should be underscored: (a) the more severe status of cases treated with IgGAM than comparators as reflected by the higher SOFA score and the prevalence of multiple organ dysfunction syndrome; and (b) the lack of heterogeneity because in all treated cases of our study, IgGAM was started within the first 24 h from signs of infection. This provides homogeneity in the analysed cohort, which is missing in previous published trials where no precise time limit between start of signs of infection and start of IgGAM treatment applied [15]. The importance of time delay in the start of IgGAM has also been shown in one retrospective analysis of 129 patients; survivors were started on IgGAM treatment earlier than non-survivors (23 versus 63 h) [16].

Our results corroborate one analysis of 57 cases of septic shock treated with IgGAM and another 57 propensity-matched non-treated comparators. Treatment with IgGAM was associated with significant survival benefit. However, this study failed to report on the impact of microbiology of treated cases and of co-administered antimicrobial treatment [17].

The present study provides promising data supporting the use of polyclonal IgM-enriched immunoglobulin preparations as adjunctive of antimicrobial treatment for the management of severe infections caused by MDR Gram-negative bacteria. These pathogens progressively emerge in critically ill patients, causing infections with reported mortality even exceeding 50% [18,19]. A prospective randomized trial is warranted to prove the role of polyclonal IgM-enriched preparations as an adjunctive therapeutic strategy for severe infections by MDR Gram-negative bacteria.

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Transparency declaration

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Appendix A. Supplementary material

Additional Supporting Information may be found in the online version of this article at http://dx.doi.org/10.1016/j.cmi.2016.01.021.

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