Doctor of Philosophy

Impact of Metformin Exposure During *Staphylococcus aureus* Bacteremia in Patients With Diabetes Mellitus

당뇨병이 있는 황색포도알균 균혈증 환자에서 metformin 사용이 미치는 영향에 대한 분석

The Graduate School

of the University of Ulsan

Department of Medicine

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Impact of Metformin Exposure During *Staphylococcus aureus* Bacteremia in Patients With Diabetes Mellitus

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A Dissertation

Submitted to
the Graduate School of the University of Ulsan
In partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

by

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Department of Medicine
University of Ulsan, Korea
February 2022

Impact of Metformin Exposure During *Staphylococcus aureus* Bacteremia in Patients With Diabetes Mellitus

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ABSTRACT

Background: Increasing evidence has suggested that metformin may play positive roles in a wide range of infectious diseases. This study aimed to investigate the impact of metformin exposure during *Staphylococcus aureus* bacteremia (SAB) in patients with diabetes.

Methods: A 3-year observational cohort study of 452 patients (aged ≥16 years) with SAB was performed at a tertiary care hospital. Metformin exposure was defined as receiving metformin during SAB, regardless of metformin use before the onset of bacteremia. The patients were divided into three groups based on diabetes status and metformin exposure. I compared the clinical features and outcomes and evaluated factors associated with mortality. A microbiological study was also conducted. Inflammatory mediators (IFN-γ, IL-6, and IL-10) were measured in plasma samples of the patients with SAB. Host immune responses were examined in healthy blood donors by *in vitro* activation of PBMC following exposures to *S. aureus* and metformin.

Results: Of 452 patients, 51 (11.3%) were classified in Group A (diabetes with metformin exposure), 115 (25.4%) in Group B (diabetes without metformin exposure), and 286 (63.3%) in Group C (no diabetes). The 30-day mortality rate in Group A was significantly lower than that in Group B (3.9% versus 14.8%, $P = 0.04$) and lower than that in Group C (3.9% versus 17.1%, $P = 0.02$). The mortality rates did not differ between Group B and Group C (14.8% versus 17.1%, $P = 0.57$). The rates of persistent and recurrent bacteremia were comparable among the three groups. Multivariate analysis indicated that metformin exposure was significantly associated with reduced mortality (adjusted odds ratio, 0.20; 95% confidence interval, 0.04–0.88; $P = 0.03$). The microbiological characteristics were similar among the three groups. The plasma cytokine levels of 68 patients with SAB were measured. Compared

with group C, the median IFN-γ (0 versus 0 pg/ml; $P = 0.02$), IL-6 (5.3 versus 20.7 pg/ml; $P = 0.003$), and IL-10 (0.3 versus 2.0 pg/ml; $P = 0.02$) levels were significantly lower in group A. The median cytokine levels were similar between group A and group B and between group B and group C. Changes in the immune cell population in ten healthy blood donors were identified. The median percentage of monocytes (0.09% versus 0.57%, $P = 0.02$) and Th2 cells (0% versus 0.74%, $P = 0.04$) was significantly lower in *S. aureus* and metformin-stimulated group than that of control group. Compared with stimulating *S. aureus* only, exposure to *S. aureus* and metformin resulted in no changes in the immune cell population. Measurement of IFN-γ levels were available in five healthy blood donors. There was a non-significant trend toward higher IFN-γ levels in *S. aureus* only-stimulated group than control group ($P = 0.07$ by Wilcoxon signed-rank test). Compared with *S. aureus* only-stimulated group, the production of IFN-γ was significantly increased after exposure to *S. aureus* and metformin ($P = 0.04$ by Wilcoxon signed-rank test).

Conclusions: Metformin exposure during SAB appears to be an independent predictor of survival among patients with diabetes. Given the novel immunomodulatory roles of metformin as well as its well-established efficacy, good safety profile, and relatively low cost, further exploration is warranted to repurpose metformin as a host-directed therapy.

Keywords: *Staphylococcus aureus*; bacteremia; diabetes mellitus; metformin; cytokine; immune response.

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INTRODUCTION

Staphylococcus aureus remains a leading cause of bloodstream infections in both community and healthcare settings, and *S. aureus* bacteremia (SAB) is associated with significant morbidity and mortality.[1)] Patients with diabetes mellitus (DM) may have increased susceptibility to *S. aureus* colonization and infection compared with the general population, and DM has been identified as an independent risk factor for developing SAB.[2, 3)] Several cohort studies have shown the prevalence of diabetes in patients with SAB to be substantial, with varying rates (20%–40%) among populations.[4)]

Metformin, a widely used first-line oral antidiabetic drug for type 2 DM, has recently received increasing attention as a potential anti-infective agent.[5)] Although the mechanisms underlying the beneficial effects of metformin beyond its glucose-lowering action are not fully understood, a number of laboratory and clinical studies have suggested that metformin may have protective and therapeutic roles in a wide range of infectious diseases.[5)] A recent meta-analysis of five retrospective cohort studies indicated that metformin use might be associated with lower mortality in patients with sepsis.[6)]

There are few *in vitro* and animal studies that have investigated the effects of metformin on the pathogenesis of *S. aureus* infection, showing that metformin inhibits glucose-mediated bacterial growth in airway epithelium.[7)] However, to my knowledge, no human studies evaluating the association between metformin and SAB have been published. Therefore, I conducted a cohort study to evaluate the impact of metformin exposure during SAB in patients with DM. In addition, an *in vitro* assessment of host immune responses following exposures to *S. aureus* and metformin was performed using blood samples of patients with SAB and healthy blood donors.

MATERIALS AND METHODS

Study design and patient selection

This observational cohort study was performed at the Asan Medical Center, a 2700-bed tertiary care teaching hospital in Seoul, Republic of Korea. From January 2016 through December 2018, patients aged ≥16 years with SAB were enrolled and followed up according to the study protocol over 12 weeks. Only the first episode of SAB in each patient was included in the analysis. Patients with polymicrobial bacteremia were excluded. The study was approved by the Asan Medical Center Institutional Review Board.

Data collection and definitions

The study data were derived from a prospective registry-based SAB cohort. All medical records were reviewed using standardized study protocols. Demographic characteristics, underlying diseases or conditions, laboratory results, site of infection, patient management, and clinical outcomes were evaluated. Patient-reported information about antidiabetic medication history during the previous month in electronic medical records was collected retrospectively. Additionally, I used primary care prescription records provided by referring physicians.

Metformin exposure was defined as receiving metformin or metformin-containing drugs during SAB, regardless of metformin use before the onset of bacteremia. Site of acquisition was classified as community-onset (community-associated or healthcare-associated) and nosocomial, as previously described.[8] Site of infection was determined based on clinical, radiological, and microbiological investigations. Empirical antibiotic therapy was considered inappropriate if an antibiotic given within 24 hours of the index blood culture was not active against the isolated organism. Persistent bacteremia was defined as bacteremia for ≥7 days while receiving appropriate antibiotic therapy. Recurrent bacteremia was defined as a

subsequent episode of bacteremia within 30 days after discontinuation of antibiotic therapy. The primary outcome was 30-day all-cause mortality.

Microbiological data

All *S. aureus* isolates were identified using standard methods. Antimicrobial susceptibilities were determined using the MicroScan system (Dade Behring, West Sacramento, CA, USA) and the standard criteria of the Clinical and Laboratory Standards Institute. Methicillin resistance was confirmed by polymerase chain reaction detection of the *mecA* gene. The minimum inhibitory concentration (MIC) of vancomycin was determined using the Etest (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. Staphylococcal cassette chromosome *mec* (SCC*mec*) type, multilocus sequence type (MLST), and *agr* genotype were identified using previously described methods.[9-11] Clonal complexes (CCs) were assigned to groups of isolates sharing six of seven alleles by use of eBURST (http://eburst.mlst.net).

Cytokine measurements

Inflammatory mediators were measured in plasma samples of participants, who provided informed consent for blood sample collection, using ProcartaPlex Multiplex Immunoassays (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions (Luminex^{TM} 200^{TM} System, Thermo Fisher Scientific, Waltham, MA, USA). Circulating concentrations of interferon (IFN)-γ, interleukin (IL)-6, and IL-10 in each patient was included in the analysis. Secreted IFN- γ in culture supernatants were quantified by in-house sandwich enzyme-linked immunosorbent assays (ELISAs).

***In vitro* activation of PBMC**

Peripheral blood mononuclear cells (PBMC) were thawed and washed before counting.

Cells were re-suspended to a final concentration of 0.5–2 × 10^6 cells/ml in cell culture medium (RPMI-1640 supplemented with 20 mM HEPES, penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM) (all from HyClone Laboratories Inc) and 10% heat-inactivated fetal calf serum (Gibco by Life Technologies). The cells were added to flat-bottomed 96-well plates (Costar, Cambridge, UK) and incubated for 48 hours at 37 °C in 5% CO2 atmosphere with cell alone as negative control, with heat-killed *S. aureus* or with 1 mM metformin (Sigma Aldrich). Cell culture supernatants were collected for cytokine analysis by ELISA.

Flow cytometry

Cultured cells were washed twice in fluorescence-activated cell sorting buffer (PBS containing 0.1% sodium azide and 0.2% bovine serum albumin) and incubated with a viability dye for 30 minutes. Cells were incubated for 20 minutes with pooled human immunoglobulin G to block nonspecific antibody binding (FcX solution; BioLegend), and surface staining was performed using fluorescently conjugated antibodies CD3-Amcyan, CD4-FITC, CD8-PerCP-cy5.5, CD56-APC, CD14-Pacific Blue, CCR4-PE, CD127-PerCP-Cy5.5, CD25-APC, CCR6-Pacific Blue, CD45RO-PE-Cy7. Flow cytometry data were acquired on a BD FACSCanto II (BD Biosciences, San Jose, CA, USA) and analyzed with FlowJo software (FlowJo, Ashland, OR, USA).

Statistical analysis

Categorical variables were analyzed using the chi-square or Fisher's exact test, and continuous variables were analyzed using Student's *t*-test, the Mann-Whitney *U* test, or Kruskal-Wallis test, as appropriate. The median differences of paired samples were assessed with the use of the Wilcoxon signed-rank test. Risk factors associated with mortality were assessed using multivariate logistic regression analysis. All variables with statistical

significance in the univariate analysis were included in the multivariate analysis. The final model was constructed using the backward elimination method. Survival analysis was conducted using the Kaplan-Meier method, and 30-day cumulative survival was compared using the log-rank test. All statistical analyses were performed using SPSS for Windows, version 25.0 (IBM Corp., Armonk, NY, USA), with $P < 0.05$ considered statistically significant.

RESULTS

Study population and patient characteristics

A total of 452 patients with SAB were identified during the study period. Of these, 51 (11.3%) were classified in Group A (diabetes with metformin exposure), 115 (25.4%) in Group B (diabetes without metformin exposure), and 286 (63.3%) in Group C (no diabetes) (Fig. 1).

The baseline characteristics of these patients are shown in Table 1. The median age was 64 years (interquartile range [IQR], 54–72 years), and 262 (58.0%) were male. The site of acquisition of bacteremia was classified as community-associated (*n* = 87 [19.2%]), healthcare-associated (*n* = 179 [39.6%]), and nosocomial (*n* = 186 [41.2%]). Hypertension (*n* = 192 [42.5%]) was the most common underlying comorbidity, followed by cancer (*n* = 182 [40.3%]), diabetes (*n* = 166 [36.7%]), immunosuppressive therapy (*n* = 123 [27.2%]), liver cirrhosis (*n* = 73 [16.2%]), chronic kidney disease (*n* = 59 [13.1%]), hematologic malignancy (*n* = 32 [7.1%]), heart failure (*n* = 30 [6.6%]), solid organ transplantation (*n* = 27 [6.0%]), and neutropenia (*n* = 20 [4.4%]). The primary sites of infection were as follows: catheter-associated (24.3%), osteoarticular (11.7%), skin and soft tissue (10.0%), endovascular (9.5%), and unknown (17.5%). Age, gender distribution, Charlson Comorbidity Index, APACHE II score, as well as rates of methicillin resistance, hypertension, liver cirrhosis, chronic kidney disease, heart failure, septic shock, and skin and soft tissue infection, were significantly different among the three groups. Body mass index (BMI) and serum lactate concentration at the onset of bacteremia were comparable among the three groups. The mean glycosylated hemoglobin (HbA1c) level was significantly higher in Group A than in Group B (8.1% versus 7.0%, *P* = 0.03). The rate of insulin use during SAB was similar between Group A and Group B (58.8% versus 70.4%, *P* = 0.14). The receipt of inappropriate empirical antibiotic therapy was not different among the three groups.

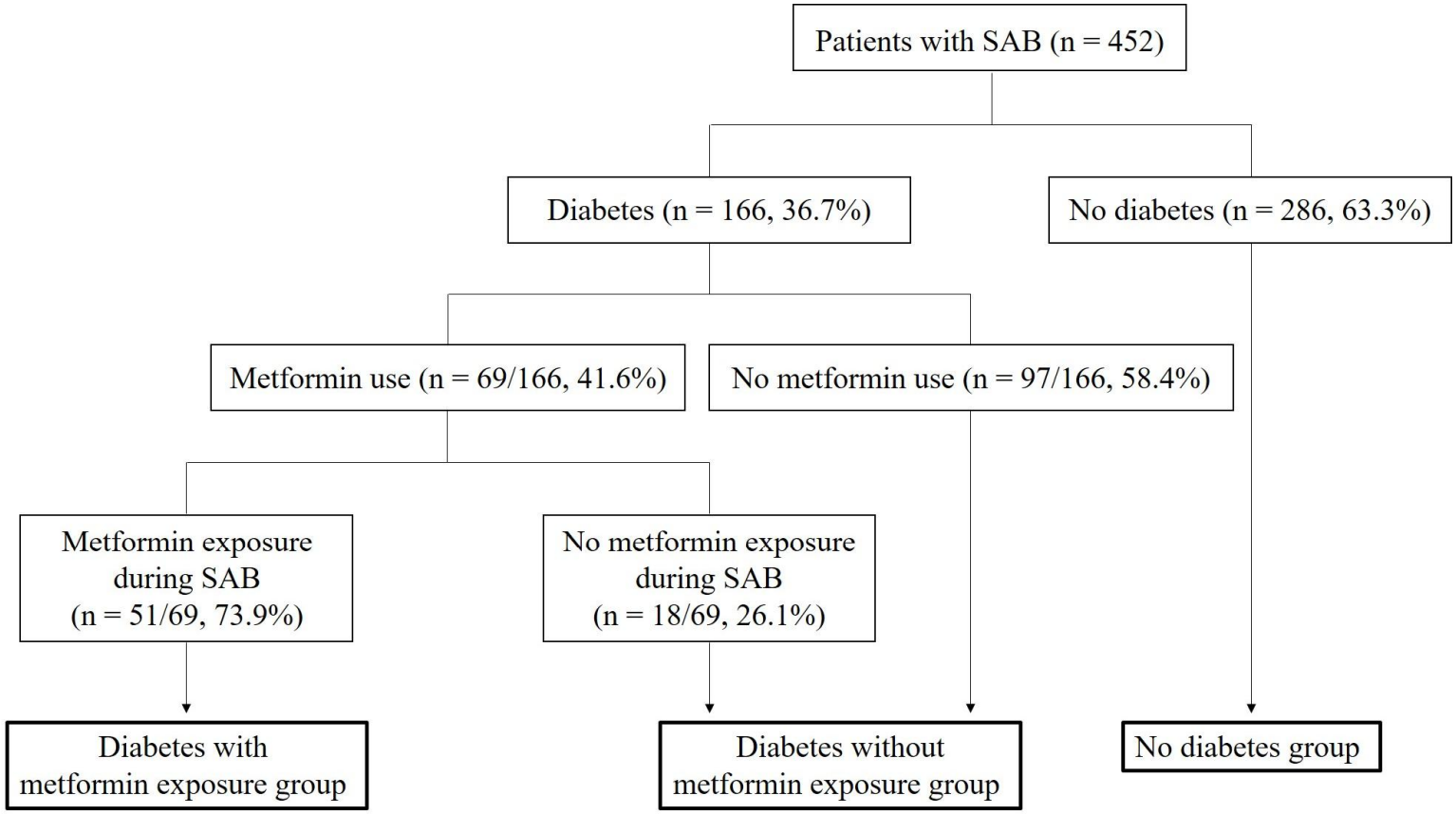

Fig. 1. Flow diagram of the study. SAB, *Staphylococcus aureus* bacteremia.

Table 1. Baseline characteristics of 452 patients with *S. aureus* bacteremia stratified by diabetes status and metformin exposure

Characteristic	Group A (n = 51) Diabetes with metformin exposure	Group B (n = 115) Diabetes without metformin exposure	Group C (n = 286) No diabetes	*P* value
Age, median (IQR)	67 (61-74)	66 (59-73)	62 (48-70)	<0.001
Male	31 (60.8)	79 (68.7)	152 (53.1)	0.02
Methicillin resistance	26 (51.0)	67 (58.3)	119 (41.6)	0.009
Site of acquisition				
Community-associated	14 (27.5)	15 (13.0)	58 (20.3)	0.07
Healthcare-associated	24 (47.1)	50 (43.5)	105 (36.7)	0.23
Nosocomial-onset	13 (25.5)	50 (43.5)	123 (43.0)	0.05
Comorbidity				
Hypertension	32 (62.7)	72 (62.6)	88 (30.8)	<0.001
Cancer	17 (33.3)	38 (33.0)	127 (44.4)	0.06
Immunosuppressive therapy	10 (19.6)	34 (29.6)	79 (27.6)	0.40
Liver cirrhosis	2 (3.9)	20 (17.4)	51 (17.8)	0.04
Chronic kidney disease	1 (2.0)	33 (28.7)	25 (8.7)	<0.001
Hematologic malignancy	4 (7.8)	5 (4.3)	23 (8.0)	0.42
Heart failure	4 (7.8)	16 (13.9)	10 (3.5)	0.001
Solid organ transplantation	3 (5.9)	11 (9.6)	13 (4.5)	0.16
Neutropenia (ANC <500/μL)	0 (0)	5 (4.3)	15 (5.2)	0.24
Charlson comorbidity index, median (IQR)	2 (2-4)	3 (3-5)	2 (1-4)	<0.001
APACHE II, median (IQR)	12 (9-16)	18 (14-22)	15 (10-19)	<0.001

Table 1. Continued

Septic shock	0 (0)	14 (12.2)	37 (12.9)	0.03
BMI (kg/m^2), median (IQR)[a]	22 (20-24)	23 (19-25)	22 (19-25)	0.89
Lactate (mmol/L), median (IQR)[a]	1.8 (1.1-2.8)	1.4 (1.2-2.5)	1.5 (1.1-2.5)	0.82
HbA1c (%), mean ± SD[b]	8.1 ± 2.2	7.0 ± 1.4	NA	0.03
Insulin use	30 (58.8)	81 (70.4)	NA	0.14
Primary site of infection				
Catheter-associated	10 (19.6)	26 (22.6)	74 (25.9)	0.56
Osteoarticular	4 (7.8)	9 (7.8)	40 (14.0)	0.15
Skin and soft tissue	10 (19.6)	20 (17.4)	15 (5.2)	<0.001
Endovascular	1 (2.0)	13 (11.3)	29 (10.1)	0.14
Unknown	10 (19.6)	14 (12.2)	55 (19.2)	0.22
Metastatic infection	7 (13.7)	21 (18.3)	60 (21.0)	0.45
Prosthetic device	9 (17.6)	29 (25.2)	45 (15.7)	0.09
Removal of eradicable foci	23 (99.5)	50 (80.6)	121 (87.7)	0.39
Inappropriate empirical antibiotic therapy	14 (27.5)	27 (23.5)	48 (16.8)	0.11
Duration of bacteremia, median (IQR)	1 (1-3)	1 (1-3)	1 (1-3)	0.69

Data are presented as No. (%) of patients unless otherwise indicated.

Abbreviations: ANC, absolute neutrophil count; APACHE, acute physiology and chronic health evaluation; BMI, body mass index; HbA1c, hemoglobin A1c; IQR, interquartile range; NA, not applicable; SD, standard deviation.

[a] BMI and serum lactate data at the onset of bacteremia were available for 92.9% (420/452) and 51.3% (232/452) of all patients, respectively.

[b] Serum HbA1c data at the onset of bacteremia were available for 28.3% (47/166) of diabetic patients.

Clinical outcomes

Fig. 2 shows the 30-day mortality, persistent bacteremia, and recurrent bacteremia rates of 452 patients with SAB. A total of 68 patients died, resulting in a crude mortality rate of 15.0%. The mortality rate in Group A was significantly lower than that in Group B (3.9% versus 14.8%, $P = 0.04$) as well as that in Group C (3.9% versus 17.1%, $P = 0.02$). Mortality rates did not differ between Group B and Group C (14.8% versus 17.1%, $P = 0.57$). The rates of persistent and recurrent bacteremia were comparable among the three groups.

The Kaplan-Meier survival analysis showed significant differences in 30-day cumulative survival between Group A and Group B ($P = 0.046$) and between Group A and Group C ($P = 0.02$) (Fig. 3).

Risk factors associated with 30-day mortality

The risk factors associated with 30-day mortality in 452 patients with SAB are shown in Table 2. In the univariate analysis, age, cancer, liver cirrhosis, Charlson Comorbidity Index, APACHE II score, septic shock, serum lactate concentration, and metformin exposure were identified as significant variables associated with mortality. The methicillin resistance rates were not different between patients who survived and those who died (46.9% [180/384] versus 47.1% [32/68]; $P = 0.98$). DM was not associated with an increased risk of death. Multivariate analysis indicated that Charlson Comorbidity Index (adjusted odds ratio [aOR], 158 1.23; 95% confidence interval [CI], 1.08–1.40; $P = 0.001$), APACHE II score (aOR, 1.06; 95% CI, 1.02–1.10; $P = 0.004$), and metformin exposure (aOR, 0.20; 95% CI, 0.04–0.88; $P = 0.03$) were significantly associated with mortality.

Microbiological findings

The microbiological characteristics of the 452 *S. aureus* isolates are summarized in Table 3. Six CCs accounted for 93.2% of isolates (CC8 [42.0%], CC5 [30.1%], CC1 [10.6%],

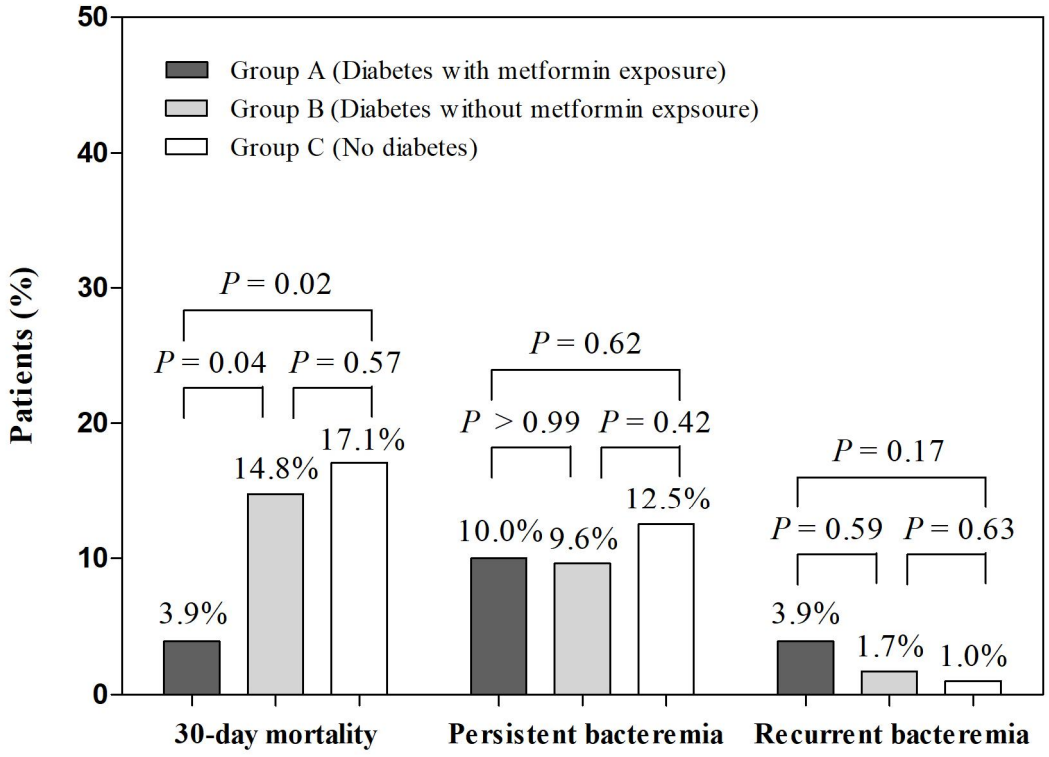

Fig. 2. Clinical outcomes of 452 patients with *S. aureus* bacteremia stratified by diabetes status and metformin exposure.

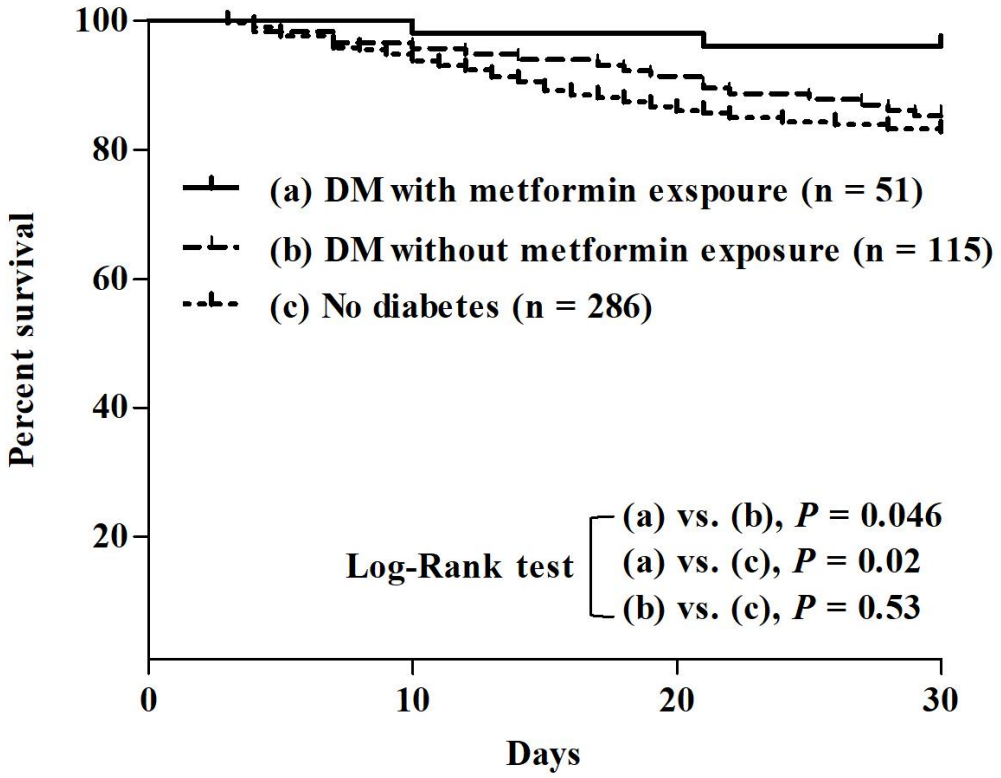

Fig. 3. Kaplan-Meier survival curves, at 30 days, for the 452 patients with *S. aureus* bacteremia stratified by diabetes status and metformin exposure. DM, diabetes mellitus.

Table 2. Univariate and multivariate analyses of risk factors associated with 30-day mortality in 452 patients with *S. aureus* bacteremia

			Univariate analysis		Multivariate analysis[c]	
Risk factor	Survival (n = 384)	Death (n = 68)	OR (95% CI)	*P* value	aOR (95% CI)	*P* value
Age, median (IQR)	63 (53-71)	67 (57-75)	1.02 (1.00-1.04)	0.046	1.02 (1.00-1.05)	0.04
Male	227 (59.1)	35 (51.5)	0.73 (0.44-1.23)	0.24		
Methicillin resistance	180 (46.9)	32 (47.1)	1.01 (0.60-1.69)	0.98		
Site of acquisition						
Community-associated	79 (20.6)	8 (11.8)	0.52 (0.24-1.12)	0.09		
Healthcare-associated	145 (37.8)	34 (50.0)	1.65 (0.98-2.77)	0.06		
Nosocomial-onset	160 (41.7)	26 (38.2)	0.87 (0.51-1.47)	0.60		
Comorbidity						
Hypertension	162 (42.2)	29 (42.6)	1.02 (0.61-1.72)	0.94		
Cancer	141 (36.7)	41 (60.3)	2.62 (1.54-4.44)	<0.001	1.82 (0.97-3.42)	0.06
Diabetes mellitus	147 (38.3)	19 (27.9)	0.63 (0.35-1.10)	0.10		
Immunosuppressive therapy	98 (25.5)	25 (36.8)	1.70 (0.99-2.92)	0.06		
Liver cirrhosis	56 (14.6)	17 (25.0)	1.95 (1.05-3.62)	0.03	…	
Chronic kidney disease	53 (13.8)	6 (8.8)	0.60 (0.25-1.47)	0.26		
Hematologic malignancy	31 (8.1)	1 (1.5)	0.17 (0.02-1.27)	0.07		
Heart failure	27 (7.0)	3 (4.4)	0.61 (0.18-2.07)	0.60		
Solid organ transplantation	25 (6.5)	2 (2.9)	0.44 (0.10-1.88)	0.40		
Neutropenia (ANC <500/μL)	17 (4.4)	3 (4.4)	1.00 (0.28-3.50)	>0.99		
Charlson comorbidity index, median (IQR)	2 (1-4)	4 (2-6)	1.30 (1.17-1.44)	<0.001	1.23 (1.08-1.40)	0.001
APACHE II, median (IQR)	15 (10-19)	16 (13-23)	1.07 (1.03-1.11)	<0.001	1.06 (1.02-1.10)	0.004
Septic shock	38 (9.9)	13 (19.1)	2.15 (1.08-4.30)	0.03	…	

Table 2. Continued

BMI (kg/m2), median (IQR)[a]	23.0 (20.0-25.7)	22.7 (19.8-25.9)	0.97 (0.91-1.03)	0.52		
Lactate (mmol/L), median (IQR)[a]	1.4 (1.1-2.2)	2.5 (1.4-3.8)	1.46 (1.22-1.76)	<0.001	…	
HbA1c (%), median (IQR)[b]	7.3 (6.3-8.2)	7.3 (6.1-8.1)	0.91 (0.49-1.70)	0.94		
Metformin exposure	49 (12.8)	2 (2.9)	0.21 (0.05-0.87)	0.02	0.20 (0.04-0.88)	0.03
Insulin use	98 (25.5)	13 (19.1)	0.69 (0.36-1.32)	0.26		
Primary site of infection						
Catheter-associated	96 (25.0)	14 (20.6)	0.78 (0.41-1.46)	0.44		
Osteoarticular	43 (11.2)	10 (14.7)	1.37 (0.65-2.87)	0.41		
Skin and soft tissue	42 (10.9)	3 (4.4)	0.38 (0.11-1.25)	0.10		
Endovascular	38 (9.9)	5 (7.4)	0.72 (0.27-1.91)	0.51		
Unknown	68 (17.7)	11 (16.2)	0.90 (0.45-1.80)	0.76		
Metastatic infection	72 (18.8)	16 (23.5)	1.33 (0.72-2.47)	0.36		
Prosthetic device	74 (19.3)	9 (13.2)	0.64 (0.30-1.35)	0.24		
Removal of eradicable foci	171 (85.5)	23 (88.5)	1.30 (0.37-4.61)	>0.99		
Inappropriate empirical antibiotic therapy	75 (19.5)	14 (20.6)	1.07 (0.56-2.03)	0.84		
Duration of bacteremia, median (IQR)	1 (1-3)	1 (1-5)	1.01 (0.97-1.05)	0.78		

Data are presented as No. (%) of patients unless otherwise indicated.

Abbreviations: ANC, absolute neutrophil count; aOR, adjusted odds ratio; APACHE, acute physiology and chronic health evaluation; CI, confidence interval; IQR, interquartile range; NE, not entered; OR, odds ratio.

[a] BMI and serum lactate data at the onset of bacteremia were available for 92.9% (420/452) and 51.3% (232/452) of all patients, respectively.

[b] Serum HbA1c data at the onset of bacteremia were available for 28.3% (47/166) of diabetic patients.

[c] Age, cancer, liver cirrhosis, Charlson comorbidity index, APACHE II score, septic shock, and metformin exposure were included in the multiple logistic regression model. Serum lactate concentration was excluded from the analysis due to missing data.

Table 3. Microbiological characteristics of 452 *S. aureus* isolates stratified by diabetes status and metformin exposure

Characteristic	Group A (n = 51) Diabetes with metformin exposure	Group B (n = 115) Diabetes without metformin exposure	Group C (n = 286) No diabetes	*P* value
Clonal complex				
CC8	23 (45.1)	51 (44.3)	116 (40.6)	0.70
CC5	15 (29.4)	34 (29.6)	87 (30.4)	0.98
CC1	6 (11.8)	8 (7.0)	34 (11.9)	0.34
CC15	1 (2.0)	3 (2.6)	17 (5.9)	0.22
CC30	1 (2.0)	3 (2.6)	12 (4.2)	0.60
CC97	1 (2.0)	5 (4.3)	5 (1.7)	0.30
MLST				
ST72	18 (35.3)	42 (36.5)	94 (32.9)	0.77
ST5	13 (25.5)	30 (26.1)	62 (21.7)	0.59
ST188	3 (5.9)	4 (3.5)	24 (8.4)	0.20
ST8	4 (7.8)	4 (3.5)	16 (5.6)	0.48
ST15	1 (2.0)	3 (2.6)	16 (5.6)	0.28
SCC*mec*[a]				
I	0/26 (0)	0/66 (0)	2/116 (1.7)	0.45
II	9/26 (34.6)	33/66 (50.0)	54/116 (46.6)	0.41
III	2/26 (7.7)	0/66 (0)	0/116 (0)	0.001
IV	15/26 (57.7)	33/66 (50.0)	60/116 (51.7)	0.80
agr subgroup				
I	33 (64.7)	70 (60.9)	180 (62.9)	0.88
II	11 (21.6)	31 (27.0)	73 (25.5)	0.76
III	3 (5.9)	4 (3.5)	18 (6.3)	0.53
IV	0 (0)	1 (0.9)	0 (0)	0.23
agr dysfunction	19 (37.3)	51 (45.1)	110 (38.5)	0.43
Vancomycin MIC by Etest				
≤1.0 mg/L	22 (43.1)	37 (32.2)	126 (44.1)	0.09
1.5 mg/L	27 (52.9)	61 (53.0)	128 (44.8)	0.24
≥2.0 mg/L	2 (3.9)	17 (14.8)	32 (11.2)	0.12
Resistance to				
Clindamycin	14 (27.5)	41 (35.7)	82 (28.7)	0.35
Ciprofloxacin	16 (31.4)	48 (41.7)	29 (24.1)	0.002
Erythromycin	17 (33.3)	44 (38.3)	92 (32.2)	0.51
Fusidic acid	17 (33.3)	42 (36.5)	100 (35.0)	0.92
Gentamicin	9 (17.6)	31 (27.0)	67 (23.4)	0.42

Table 3. Continued

Rifampicin	1 (2.0)	6 (5.2)	13 (4.5)	0.63
TMP/SMX	1 (2.0)	2 (1.7)	3 (1.0)	0.79

Data are presented as No. (%) of patients unless otherwise indicated.
Abbreviations: CC, clonal complex; MIC, minimum inhibitory concentration; MLST, multilocus sequence type; SCC*mec*, staphylococcal cassette chromosome *mec*; ST, sequence type; TMP/SMX, trimethoprim/sulfamethoxazole.
[a] 26 isolates in the group A, 66 isolates in the group B, and 116 isolates in the group C were available for the analysis.

CC15 [4.6%], CC30 [3.5%], and CC97 [2.4%]). ST72 (34.1%) and ST5 (23.2%) were the major clonal types. Of the 208 isolates (46.0%) that were available for SCC*mec* data, the rate of SCC*mec* type III was significantly different among the three groups. The rates of *agr* subgroup and *agr* dysfunction were comparable among the three groups. The overall vancomycin MIC distribution was as follows: MIC ≤1.0 mg/L, 185 isolates (40.9%); MIC of 1.5 mg/L, 216 isolates (47.8%), and MIC ≥2.0 mg/L, 51 isolates (11.3%). The antimicrobial susceptibility testing to non-β-lactam antibiotics showed no differences among the three groups except for ciprofloxacin.

Cytokine analysis

A total of 138 patients (30.5%) provided informed consent. Of these, 68 (49.3% [68/138]) (18 in group A, 14 in group B, and 36 in group C) were available for cytokine analysis. The baseline characteristics of these patients are shown in Table 4. Age, Charlson Comorbidity Index, duration of bacteremia, as well as rates of methicillin resistance, nosocomial-onset, hypertension, liver cirrhosis, heart failure, skin and soft tissue infection, and metastatic infection, were significantly different among the three groups.

The circulating concentrations of IFN-γ, IL-6, and IL-10 of 68 patients with SAB are shown in Table 5 and Fig. 4. Compared with group C, the median IFN-γ (0 versus 0 pg/ml; $P = 0.02$), IL-6 (5.3 versus 20.7 pg/ml; $P = 0.003$), and IL-10 (0.3 versus 2.0 pg/ml; $P = 0.02$) levels were significantly lower in group A. The median cytokine levels were similar between group A and group B and between group B and group C. The median time from SAB to blood sampling was comparable among the three groups.

Host immune responses in healthy blood donors

I further examined the host immune responses in ten healthy blood donors (5 males and 5 females) by *in vitro* activation of PBMC following exposures to *S. aureus* and metformin.

Table 4. Baseline characteristics of 68 patients with *S. aureus* bacteremia, whose blood samples were available for cytokine analysis, stratified by diabetes status and metformin exposure

Characteristic	Group A (n = 18) Diabetes with metformin exposure	Group B (n = 14) Diabetes without metformin exposure	Group C (n = 36) No diabetes	*P* value
Age, median (IQR)	67 (61-69)	53 (48-63)	59 (48-68)	0.015
Male	11 (61.1)	11 (78.6)	25 (69.4)	0.57
Methicillin resistance	5 (27.8)	10 (71.4)	14 (38.9)	0.04
Site of acquisition				
Community-associated	9 (50.0)	12 (14.3)	13 (36.1)	0.11
Healthcare-associated	9 (50.0)	4 (28.6)	13 (36.1)	0.43
Nosocomial-onset	0 (0)	8 (57.1)	10 (27.8)	0.001
Comorbidity				
Hypertension	11 (61.1)	6 (42.9)	8 (22.2)	0.02
Cancer	7 (38.9)	5 (35.7)	16 (44.4)	0.83
Immunosuppressive therapy	2 (11.1)	5 (35.7)	7 (19.4)	0.23
Liver cirrhosis	1 (5.6)	7 (50.0)	11 (30.6)	0.02
Chronic kidney disease	0 (0)	3 (21.4)	4 (11.1)	0.14
Hematologic malignancy	3 (16.7)	1 (7.1)	2 (5.6)	0.39
Heart failure	0 (0)	2 (14.3)	0 (0)	0.02
Solid organ transplantation	0 (0)	2 (14.3)	2 (5.6)	0.23
Neutropenia (ANC <500/μL)	0 (0)	1 (7.1)	0 (0)	0.14
Charlson comorbidity index, median (IQR)	3 (2-4)	4 (3-5)	2 (1-3)	0.003
APACHE II, median (IQR)	10 (8-14)	14 (10-21)	14 (8-19)	0.18

Table 4. Continued

Septic shock	0 (0)	2 (14.3)	5 (13.9)	0.25
BMI (kg/m^2), median (IQR)[a]	24 (19-25)	24 (22-28)	23 (20-27)	0.96
Lactate (mmol/L), median (IQR)[a]	1.7 (1.0-2.1)	1.7 (1.2-3.4)	1.6 (1.3-2.3)	0.90
HbA1c (%), median (IQR)[b]	8.1 (6.9-10.1)	6.9 (5.7-9.8)	NA	0.28
Insulin use	8 (44.4)	10 (71.4)	NA	0.13
Primary site of infection				
Catheter-associated	3 (16.7)	5 (35.7)	7 (19.4)	0.37
Osteoarticular	2 (11.1)	0 (0)	9 (25.0)	0.08
Skin and soft tissue	3 (16.7)	5 (35.7)	2 (5.6)	0.03
Endovascular	1 (5.6)	0 (0)	7 (19.4)	0.10
Unknown	4 (22.2)	2 (14.3)	4 (11.1)	0.55
Metastatic infection	4 (22.2)	1 (7.1)	16 (44.4)	0.02
Prosthetic device	2 (11.1)	2 (14.3)	9 (25.0)	0.41
Removal of eradicable foci	7 (70.0)	10 (90.9)	13 (86.7)	0.40
Inappropriate empirical antibiotic therapy	6 (33.3)	4 (28.6)	4 (11.1)	0.12
Duration of bacteremia, median (IQR)	1 (1-6)	1 (1-4)	6 (1-10)	0.008

Data are presented as No. (%) of patients unless otherwise indicated.

Abbreviations: ANC, absolute neutrophil count; APACHE, acute physiology and chronic health evaluation; BMI, body mass index; HbA1c, hemoglobin A1c; IQR, interquartile range; NA, not applicable; SD, standard deviation.

[a] BMI and serum lactate data at the onset of bacteremia were available for 95.6% (65/68) and 50.0% (34/68) of all patients, respectively.

[b] Serum HbA1c data at the onset of bacteremia were available for 50.0% (16/32) of diabetic patients.

Table 5. Plasma cytokine concentrations of 68 patients with *S. aureus* bacteremia stratified by diabetes status and metformin exposure

Cytokine	Group A (n = 18) Diabetes with metformin exposure	Group B (n = 14) Diabetes without metformin exposure	Group C (n = 36) No diabetes	*P* value A vs. B	*P* value A vs. C	*P* value B vs. C
IFN-γ (pg/ml), median (IQR)	0 (0-0.1)	0 (0-0)	0 (0-0)	0.24	0.02	0.77
IL-6 (pg/ml), median (IQR)	5.3 (0.7-12.3)	20.9 (2.0-36.1)	20.7 (11.1-46.6)	0.16	0.003	0.35
IL-10 (pg/ml), median (IQR)	0.3 (0.2-1.4)	1.1 (0.2-5.4)	2.0 (0.6-5.5)	0.44	0.02	0.37
Time from SAB to blood sampling, median (IQR)	6.0 (4.8-7.3)	7.5 (5.0-14.0)	6.5 (4.3-8.0)	0.10	0.49	0.15

Abbreviations: IFN, interferon; IL, interleukin; IQR, interquartile range; SAB, *S. aureus* bacteremia.

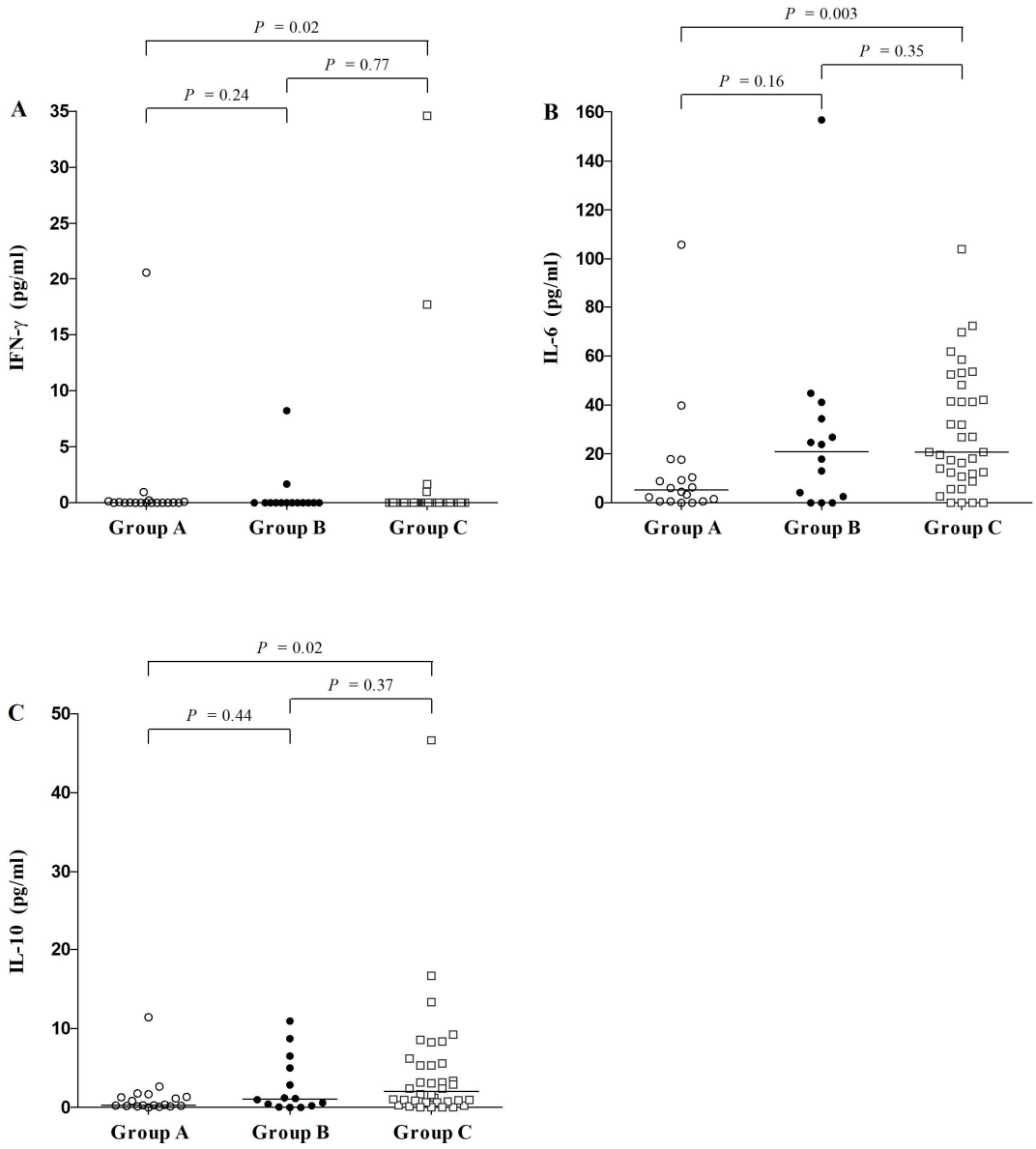

Fig. 4. Plasma cytokine concentrations of 68 patients with *S. aureus* bacteremia stratified by diabetes status and metformin exposure. Group A, Diabetes with metformin exposure. Group B, Diabetes without metformin exposure. Group C, No diabetes.

Changes in the immune cell population were identified after stimulation with metformin only, *S. aureus* only, and *S. aureus* and metformin. The median percentage of monocytes was significantly lower in *S. aureus* and metformin-stimulated group than that of control group (0.09% versus 0.57%, $P = 0.02$) (Fig. 5). The proportion of B cells, T cells, and NK cells were not different among the four groups. Comparison of the distribution of T-cell subsets are shown in Fig. 6. The median percentage of T helper 2 (Th2) cells was significantly lower in *S. aureus* and metformin-stimulated group than that of control group (0% versus 0.74%, $P = 0.04$). The proportion of CD4+ T cells, CD8+ T cells, Tregs, Th1 cells, and Th17 cells were not different among the four groups. Compared with stimulating *S. aureus* only, exposure to *S. aureus* and metformin resulted in no changes in the immune cell population.

Measurement of IFN-γ levels in supernatants of PBMC cultures in response to *S. aureus* and metformin stimuli were available in five healthy blood donors (Fig. 7). There was a non-significant trend toward higher IFN-γ levels in *S. aureus* only-stimulated group than control group ($P = 0.07$ by Wilcoxon signed-rank test). Compared with *S. aureus* only-stimulated group, the production of IFN-γ was significantly increased after exposure to *S. aureus* and metformin ($P = 0.04$ by Wilcoxon signed-rank test).

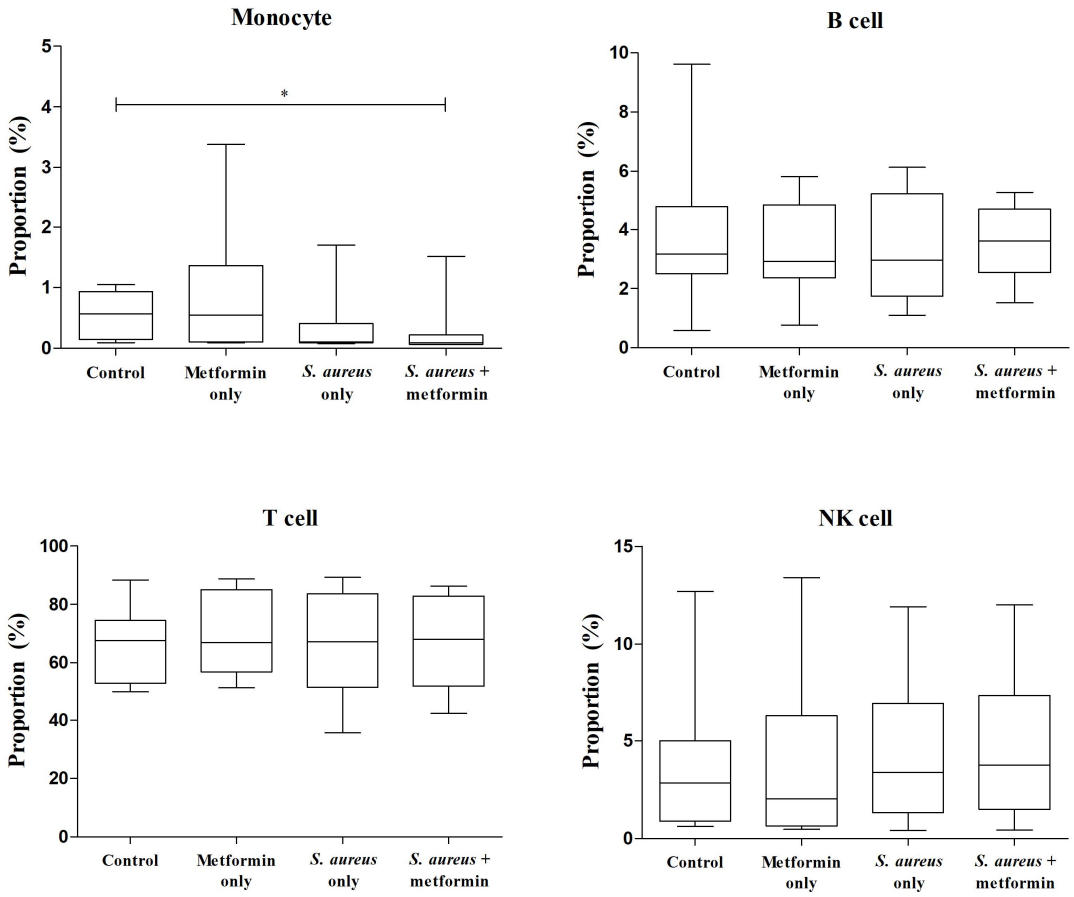

Fig. 5. Changes in the immune cell population by *in vitro* activation of PBMC after stimulation with metformin only, *S. aureus* only, and *S. aureus* and metformin in ten healthy blood donors. *$P < 0.05$.

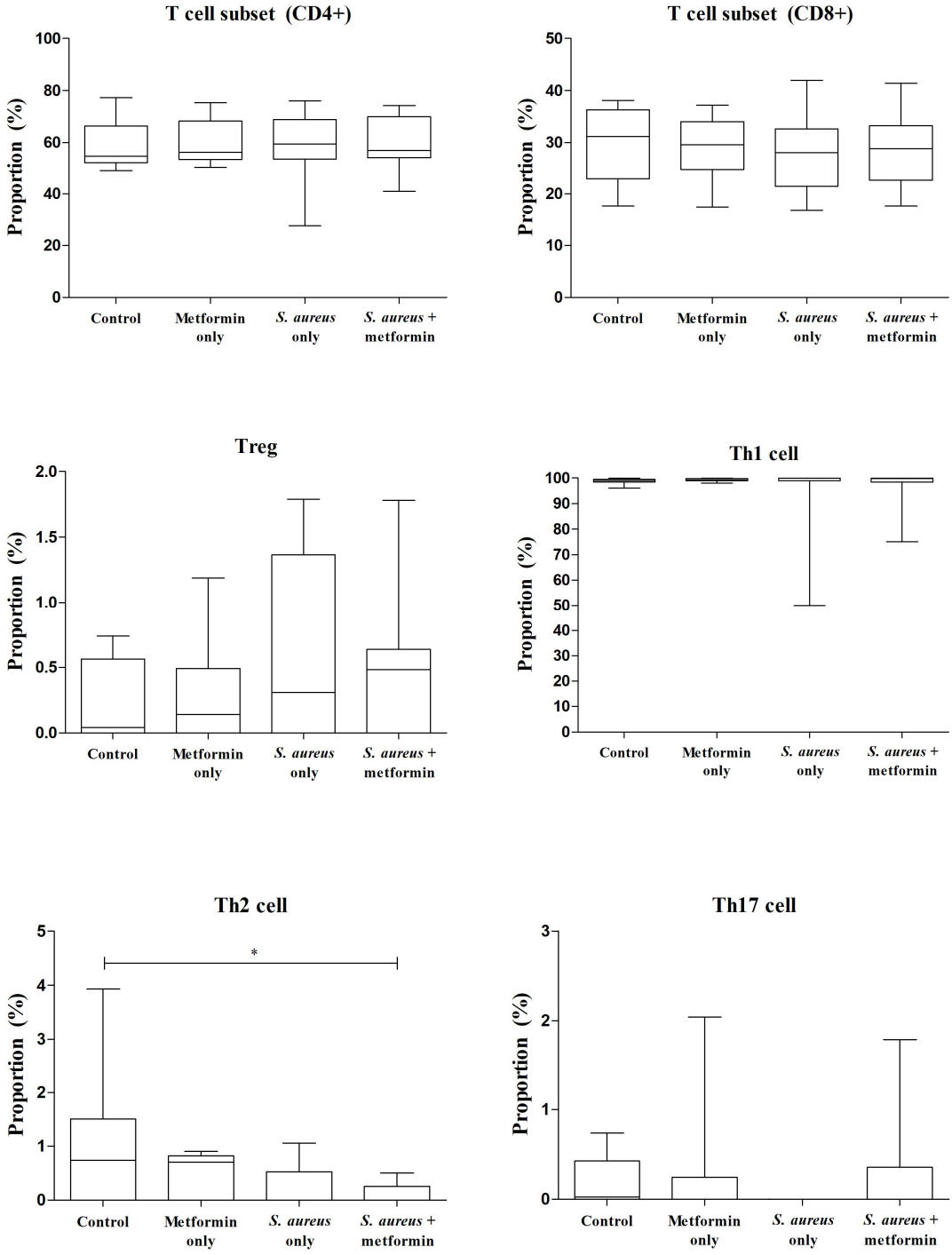

Fig. 6. Changes in T cell subsets by *in vitro* activation of PBMC after stimulation with metformin only, *S. aureus* only, and *S. aureus* and metformin in ten healthy blood donors. **P* < 0.05.

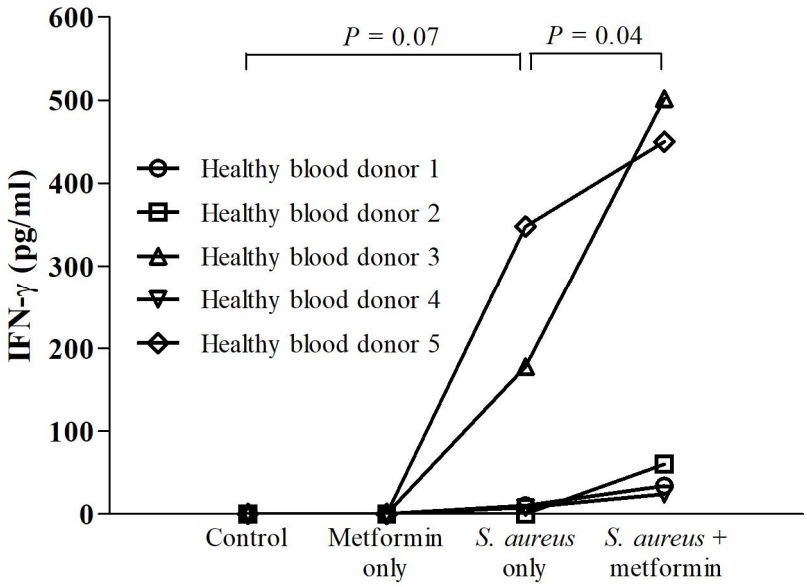

Fig. 7. IFN-γ levels in supernatants of PBMC cultures in response to *S. aureus* and metformin stimuli in five healthy blood donors. *P* values were calculated by Wilcoxon signed-rank test.

DISCUSSION

There are several reports of the possible benefits of metformin use for patients with sepsis and bacteremia.[6] However, to my knowledge, this was the first study evaluating the impact of metformin exposure during SAB on clinical outcomes in patients with DM. In this single-center observational cohort study, the overall 30-day mortality of SAB was 15.0%. More than one-third of patients had diabetes, and DM itself was not associated with an increased risk of death. Among diabetic patients, 30.7% (51/166) received metformin therapy during SAB. Most importantly, I found that metformin exposure during SAB was an independent factor for predicting survival in patients with diabetes. The mortality benefit was observed despite higher serum HbA1c levels at the onset of bacteremia in the metformin-exposed group.

Metformin, a biguanide oral hypoglycemic agent, is thought to exert its metabolic action primarily through the inhibition of hepatic gluconeogenesis.[12] Although the precise mechanisms remain ill-defined, respiratory complex I and glycerophosphate dehydrogenase are proposed as key molecular targets of metformin in mitochondria.[13, 14] More recently, the intestinal tract has been implicated as an important metformin's extrahepatic target. Metformin-induced alterations of gut microbiota composition and function may contribute to improved glucose metabolism.[15] Furthermore, accumulating evidence suggests that metformin possesses anti-infective and/or anti-inflammatory properties beyond its glucose-lowering action. The observed pleiotropic effects of metformin on various infectious and non-infectious conditions have prompted active investigations into its therapeutic applications for diabetic and non-diabetic populations.[5, 16, 17]

There are still areas of uncertainty about how metformin acts against microorganisms, and the relative contributions of glycemic control, immunomodulatory effect, and direct antimicrobial activity are not yet clear.[5] Metformin reduces the hyperglycemia-induced

proliferation of bacteria, such as *S. aureus* or *Pseudomonas aeruginosa*, in airway epithelium.[7, 18] Metformin stimulation strengthened the innate immunity of uroepithelial cells inducing enhanced extracellular and intracellular killing of *Escherichia coli*.[19] Additionally, metformin can inhibit the expression of pro-inflammatory mediators and ameliorate endotoxin-induced tissue damage associated with sepsis.[5] However, clinical studies have shown inconsistent results regarding the association between preadmission metformin use and mortality among septic patients with DM.[6] Retrospective study designs, small sample sizes, and confounding variables were considered as limitations of those studies.

Compared with sepsis studies, the present study focused on the association between metformin and SAB, and only 11.3% (51/452) of patients had septic shock. Metformin exposure was defined as receiving metformin during SAB, regardless of previous metformin use, thereby assessing the benefits of metformin therapy during bacteremia. Thus, it is not clear whether metformin use before the onset of SAB can affect outcomes. Nevertheless, I believe that my findings, combined with those of previous studies, add further evidence supporting the positive roles of metformin in bacterial bloodstream infections.

Metformin is contraindicated for patients with moderately to severely impaired kidney function because of concerns about lactic acidosis.[20] It is thus possible that the metformin non-exposed group in my study may represent patients with risk factors for poor outcomes, such as more advanced diabetes with multiple comorbidities. However, when subgroup analysis excluding the patients (n = 96) with estimated glomerular filtration rates <30 mL/min/1.73 m^2 was performed, metformin exposure (aOR, 0.19; 95% CI, 0.04–0.87; P = 0.03) remained significantly associated with reduced mortality (data not shown). This indicates that the association between metformin and SAB was not likely confounded by differences in renal function among the groups.

Interestingly, there was no significant link between DM and the risk of death from SAB in

my cohort. It is generally believed that infections are increasing in frequency and severity in diabetic patients, and these patients may have an excess risk of death owing to infection-related causes when compared with those without diabetes.[21, 22] Population-based studies have revealed DM to be associated with about a two- to three-fold increased risk of developing SAB, and the odds vary according to the type, duration, and severity of DM.[2, 3] By contrast, whether DM can contribute to poor SAB outcomes has been debated.[3, 4] A Danish group reported an increased risk of mortality after SAB in association with DM without complications but not in association with DM with complications.[3] In a pooled analysis of prospective cohort studies, DM was not associated with poorer SAB outcomes.[4] Although the reasons for the discrepant findings are not clear, my data emphasize a possible influence of metformin on SAB-associated mortality, which should be accounted for in future research.

Mechanisms of protective immunity to *S. aureus* infection in humans remain elusive.[23] *S. aureus* antigen-specific memory Th1 cells promoted monocyte/macrophage activation with increased IFN-γ production, facilitating accelerated clearance of infection in human *S. aureus* bloodstream infection.[23] Studies have suggested that selected serum cytokines, such as IL-6, IL-8, and IL-10, were associated with poor outcomes in patients with SAB and could have prognostic value of biomarkers.[24-26] Metformin is thought to attenuate immune responses through its inhibitory effect on the proinflammatory phenotype of immune cells, by inhibiting monocyte to macrophage differentiation, increasing differentiation of T cells into Treg and Th2 cells, and increasing phagocytosis of neutrophils.[16] In the present study, IL-6 and IL-10 levels were lower in metformin-exposed group than metformin non-exposed group, although this difference was significant only between group A and group C. Whether lower levels of circulating inflammatory cytokines in patients with metformin exposure are linked to anti-inflammatory effects of metformin is not clear due to differences in clinical and microbiological characteristics among the three groups. Significantly higher IFN-γ levels

were observed in *S. aureus* and metformin-stimulated group than *S. aureus* only-stimulated group in healthy blood donors. Enhanced IFN-γ responses upon *S. aureus* exposure with the addition of metformin possibly indicate the positive role of metformin in promoting T cell immune responses to *S. aureus* infection. I could not demonstrate the effects of metformin on immune cell populations in healthy blood donors.

This study had several limitations. First, it was conducted at a single tertiary care hospital in South Korea, meaning that my findings may not be generalizable to different institutions or population groups. Second, some retrospective data about previous antidiabetic medication history were included. However, as I defined metformin exposure as receiving metformin during SAB, misclassification of metformin-exposed patients as metformin non-exposed was unlikely. Third, unfortunately, I was unable to collect prescription data about classes of oral antidiabetic drugs other than metformin; neither was I able to collect data about co-medications with immunomodulatory effects, such as statins. Therefore, an assessment of the influence of those potentially significant covariates on clinical outcomes could not be performed. Fourth, detailed information about various DM-related characteristics (type, duration, complication, etc.) was unavailable in my study. Fifth, some missing values regarding patients' BMI, serum lactate, and serum HbA1c data might be sources of bias. Taken together, with other unidentified confounding factors not listed above, my findings must be interpreted with caution, and additional high-quality research is required to validate the association between metformin and SAB.

CONCLUSION

In conclusion, metformin exposure during SAB appears to be an independent factor for predicting survival among patients with diabetes. Given the novel immunomodulatory roles of metformin as well as its well-established efficacy, good safety profile, and relatively low cost, further exploration is warranted to repurpose metformin as a host-directed therapy.

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국문요약

당뇨병이 있는 황색포도알균 균혈증 환자에서 metformin 사용이 미치는 영향에 대한 분석

이주영

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의학과

배경: 제 2 형 당뇨병의 1 차 치료제로 널리 사용되는 metformin 이 다양한 감염성 질환의 치료에 긍정적인 효과를 보일 수 있다는 연구 결과들이 주목을 받고 있다. 본 연구는 당뇨병이 있는 황색포도알균 균혈증 환자에서 metformin 사용이 미치는 영향에 대해 분석하고자 하였다.

방법: 일개 3 차 병원에서 16 세 이상의 황색포도알균 균혈증 환자 452 명을 대상으로 3 년간 코호트 연구를 수행하였다. 이전 사용 여부와 관계 없이, 황색포도알균 균혈증 시기에 metformin 을 처방 받은 경우 metformin 노출로 정의하였다. 환자들은 당뇨병 여부와 metformin 노출 유무에 따라 세 그룹으로 나누어졌다. 환자들의 임상적 특징과 치료 결과를 비교하고 사망과 연관된 인자들을 분석하였다. 미생물학적 연구도 수행하였다. 황색포도알균 균혈증 환자의 혈액에서 염증성 매개체인 IFN-γ, IL-6, IL-10 을 측정하였다. 시험관 내에서 건강한 혈액 공여자의 말초혈액단핵세포를 황색포도알균과 metformin 에 노출시켜 숙주 면역반응을 조사하였다.

결과: 황색포도알균 균혈증 환자 452 명 중 51 명(11.3%)은 A 군 (metformin 에 노출된 당뇨병 환자), 115 명(25.4%)은 B 군 (metformin 에 노출되지 않은 당뇨병 환자), 그리고 286 명(63.3%)은 C 군 (당뇨병이 없는 환자)으로 분류되었다. A 군의 30 일 사망률은 B 군 (3.9% versus 14.8%, $P = 0.04$)과 C 군 (3.9% versus 17.1%, $P = 0.02$)에 비해 의미 있게 낮았다. B 군과 C 군간의 사망률 차이는 다르지 않았다 (14.8% versus 17.1%, $P = 0.57$). 지속성 그리고 재발성 균혈증 비율은 세 군간에 차이를 보이지 않았다. 다변량 분석에서 metformin 노출은 사망률 감소와 유의한 관련성을 보였다 (adjusted odds ratio, 0.20; 95% confidence interval, 0.04–0.88; $P = 0.03$). 미생물학적 특징은 세 군간에 유사하였다. 황색포도알균 균혈증 환자 68 명에서 혈액 사이토카인을 측정하였다. A 군의 IFN-γ (0 versus 0 pg/ml; $P = 0.02$), IL-6 (5.3 versus 20.7 pg/ml; $P = 0.003$), 그리고 IL-10 (0.3 versus 2.0 pg/ml; $P = 0.02$) 중앙값이 C 군에 비해 의미 있게 낮았다. A 군과 B 군, 그리고 B 군과 C 군간에는 사이토카인 값의 차이가 유사하였다. 10 명의 건강한 혈액 공여자의 혈액으로 시험관 내 면역세포 구성의 변화를 평가하였다. 황색포도알균과 metformin 동시 자극군에서 단핵구 (0.09% versus 0.57%, $P = 0.02$)와 Th2 세포 (0% versus 0.74%, $P = 0.04$) 중앙값이 대조군에 비해 의미 있게 낮았다. 그러나 황색포도알균 단일 자극군에 비해 황색포도알균과 metformin 동시 자극군의 면역세포 구성의 유의미한 변화는 보이지 않았다. 5 명의 건강한 혈액 공여자의 혈액으로 시험관 내 IFN-γ 를 측정하였다. 황색포도알균 단일 자극군에서 대조군에 비해 IFN-γ 값이 더 높은 경향을 보였으나 통계적으로 유의하진 않았다 ($P = 0.07$ by Wilcoxon signed-rank test). 황색포도알균 단일 자극군에 비해 황색포도알균과 metformin 동시 노출 시 IFN-γ 생성이 의미 있게 증가하였다 ($P = 0.04$ by Wilcoxon signed-rank test).

결론: 당뇨병이 있는 황색포도알균 균혈증 환자에서 metformin 노출은 생존의 독립적인 예후 인자일 가능성이 있다. Metformin 의 잘 알려진 효능, 약물 안전성, 상대적으로 저렴한 비용, 그리고 새롭게 알려지고 있는 면역조절 기능을 고려할 때 숙주-표적 치료제(host-directed therapy)로서의 metformin 의 역할에 대한 추가적인 연구가 필요하겠다.

중심단어: 황색포도알균; 균혈증; 당뇨병; metformin; 사이토카인; 면역반응.