Control of body temperature and immune function in patients undergoing open surgery for gastric cancer

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ABSTRACT

The influence of mild perioperative hypothermia on the immune function and incidence of postoperative wound infections has been suggested, but the specific mechanism is unclear. This study aimed to analyze the body temperature, immune function, and wound infection rates in patients receiving open surgery for gastric cancer. Body temperature was controlled in each patient using one of four different methods: wrapping limbs, head and neck; insulated blankets; warming infusion fluids and insulated blankets; and warming fluids without insulated blankets. One hundred patients were randomly divided into four groups of 25 patients each, and every group received a different intraoperative treatment for maintaining normal body temperature. Nasopharyngeal and rectal temperatures, transforming growth factor beta (TGF-β), interleukin 10 (IL-10) levels, and cluster of differentiation (CD)3$^+$ T and CD4$^+$/CD25$^+$ regulatory T cell (Treg) counts were measured before surgery and at 2 and 4 hours postoperatively. Patients were evaluated at one week after surgery for signs of infection. Intraoperative body temperature and measures of immune function varied significantly between the four groups, with the largest temperature changes observed in the group in which only the limbs were wrapped in cotton pads to control the body temperature. The lowest temperature change (i.e., close to normal temperature) and cytokine response after surgery were observed in the group in which infusion fluids and transfused blood (if needed) were heated to 37°C, peritoneal irrigation fluid was heated to 37°C, and an insulation blanket was heated to 39°C and placed under the patient. No intergroup differences were found in the infection rates at one week after surgery. In conclusion, body temperature variation during surgery affects the immune function of patients, and maintaining body temperature close to normal results in the least variation of immune function.

KEY WORDS: Gastric cancer; heating methods; infection; perioperative period; CD4$^+$ CD25$^+$ Treg; TGF-β; IL-10
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INTRODUCTION

A stable body temperature is crucial for optimal metabolism and physiological processes. This is especially important during surgery, as perioperative hypothermia may lead to detrimental complications in patients [1-4]. Thus, for achieving good operative outcomes and reducing postoperative complications, continuous monitoring of body temperature during surgery is necessary [4]. Several studies have investigated the influence of intraoperative hypothermia on the recovery phase of anesthesia as well as the impact of different methods for maintenance of normothermia, such as temperature-adjustable water mattresses or blankets and insulation blankets, on blood loss, time to extubation, time to regain consciousness, duration of post-anesthesia recovery (PAR), postoperative drainage, the incidence of arrhythmia, and postoperative bed rest time [5-10].

It has been suggested that mild perioperative hypothermia may affect the immune function of patients and increase the incidence of wound infections. For example, in peripheral blood mononuclear cells (PBMC) from patients in hypothermia group the mitogenic response was suppressed 24 and 48 hours after surgery and the production of interleukin (IL)-1β (proinflammatory cytokine) and IL-2 (growth factor for T lymphocytes) was reduced at 24 hours. Nevertheless, the underlying mechanism of how temperature affects immune response is still not clear [11]. Moreover, it was shown that mild hypothermia (33°C) induced proliferation of regulatory T cells (Tregs) that had strong immunosuppressive potential and prominent anti-inflammatory phenotype [12]. The immunosuppressive role of Tregs in immune response is well

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known, i.e., they can regulate or suppress other cells in the immune system, and in normal conditions they prevent autoimmune disease by maintaining self-tolerance [11,12]. In addition, the function of Tregs has been described in cancer; for instance, an increased frequency of cluster of differentiation (CD)$4^{+}CD25^{high}$ T cells expressing associated proteins, such as transforming growth factor (TGF)-β1 and IL-10, was shown in patients with B-cell chronic lymphocytic leukemia (CLL) [13].

Various methods for controlling body temperature and preventing hypothermia during surgery have been investigated, as well as the effect of temperature on immune response [14-23]. In our previous study, the combination of warmed infusion fluids, warmed irrigation fluids and an insulation blanket was the most effective method for maintaining body temperature during surgery [20]. Furthermore, a review of trials investigating fluid warming methods to maintain normothermia, in relation to standard care or other warming methods, suggested that better results may be obtained with the use of warmed intravenous fluids compared to room temperature intravenous fluids. However, the authors questioned whether the differences in body temperature related to different methods were clinically significant, since the intraoperative conditions or additional warming methods used may have influenced the outcomes of the usage of warmed fluids [15].

The purpose of this study was to analyze body temperature, immune function, and wound infection rates in patients receiving open surgery for gastric cancer whose body temperature was controlled with one of the four different methods: wrapping limbs, head and neck; insulated blankets; warming infusion fluids and insulated blankets; and warming fluids without insulated blankets.

MATERIALS AND METHODS

Patients

Patients scheduled for elective open surgery for gastric cancer in the period between January 2014 and June 2015 at the Department of General Surgery of the First Affiliated Hospital of Xinjiang Medical University, were recruited in this study. The inclusion criteria were: 1) diagnosis of gastric cancer confirmed with gastroscopy; 2) the American Society of Anesthesiology Physical Status (ASA PS) Class I or II; 3) normal body temperature during 3 days before the surgery; 4) patient tolerance for the insertion of a probe into the anus or mouth to measure the body temperature (the surgery did not involve the mouth or anus); 5) estimated surgical time was no longer than 5 hours; 6) patients had no immune or coagulation dysfunction; 7) the age range of patients was 59–89 years; and 8) the surgery was conducted with the patient in the supine position.

The exclusion criteria were as follows: 1) the presence of any conditions contraindicated for elective general anesthesia or those affecting basal metabolism (e.g. hypothyroidism and hyperthyroidism), as well as advanced-stage malignancies or rheumatic diseases; 2) evidence of infection or abnormal body temperature during 3 days before the surgery; 3) taking drugs known to affect immune or coagulation function, or taking any type of anti-inflammatory drugs, e.g. steroids and non-steroidal anti-inflammatory drugs (NSAIDs); 4) presence of sepsis, severe upper airway infection, lung infection, liver infection, or biliary tract infection; 5) a previous surgery in a patient was discontinued due to intraoperative hypothermia, shock, or intraoperative pathological findings; 6) the surgical method was changed due to a difference between preoperative and intraoperative diagnosis; 7) thermal insulation was discontinued due to hyperthermia, or additional thermal insulation measures were needed due to hypothermia.

The protocol for this prospective study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. A signed informed consent for the participation in the study and for surgical procedures performed was obtained from all patients.

Instruments and reagents

A blood and fluid warmer (Ryoyu Industrial Corporation, Japan), insulation blanket (Inditherm Medical, Germany), cotton pad (Xinshengbang Industry and Trade Company, Ltd., China), and ultralow-temperature freezer (Zhongke Meiling Low Temperature Technology Company, Ltd., China) were used to control body temperature during surgery. IL-10 and TGF-β were measured using enzyme-linked immunosorbent assay (ELISA) kits (Human IL-10 Platinum ELISA and Human TGF-β Platinum ELISA respectively; eBioscience, USA). All assays were performed according to the manufacturer's instructions.

Body temperature control measures

Methods for body temperature control were applied from the patient arrival to the operating room until the departure after the completion of surgery. The operating room temperature was maintained at 23°C and the humidity was 50%.

Patients were randomly assigned to 1 of 4 groups in a double-blind manner; one investigator sealed the envelope and another investigator identified the group. In Group A, both upper limbs, the lower 1/3 of lower limbs, and the head and neck were wrapped with cotton pads. In Group B, a thermal insulation blanket was placed under the patient and heated to 39°C. In Group C, infusion fluids and, if necessary, blood for transfusion were heated to 37°C, the peritoneal irrigation fluid was heated to 37°C, and an insulation blanket was heated to 39°C and placed under the patient. In Group D, infusion fluids and blood for transfusion (if necessary) were heated to 37°C,

the peritoneal irrigation fluid was heated to 37°C, but warmed insulation blankets were not used.

Anesthesia

All patients received intravenous and inhalation anesthesia. The following anesthetics were used during the surgery: imidazole (0.08 mg/kg), propofol (10.4 mg/kg), esmeron (3.25 mg/kg), remifentanil (0.04 mg/kg), dexamethasone (0.13 mg/kg), and parecoxib sodium (0.52 mg/kg).

Outcome measures

IL-10 and TGF-β levels

Peripheral venous blood (3 mL) was collected in anticoagulant tubes before anesthesia (preoperatively) and at 2 hours and 4 hours postoperatively. The blood was centrifuged, and the plasma was collected and stored at −80°C until analysis. ELISA was performed to detect the concentrations of IL-10 and TGF-β.

Measurement of $CD3^+$ T and $CD4^+CD25^+$ Treg cells

Separate samples of venous blood were collected at the time points described above, and evaluation of $CD3^+$ T and $CD4^+/CD25^+$ Treg cells was performed as previously described [13,24]. Briefly, mononuclear cells were separated using the Ficoll method and the cell density was adjusted to 2×10^6 cells/mL. Then the cell suspensions were added to 2 independent tubes (100 μL for each) into which 5 μL of CD3-FITC and 5 μL of CD4-PerCP antibodies were added, respectively. After the addition of PE-CD25 antibody (5 μL) or PE-immunoglobulin G (IgG)2a isotype control (5 μL), the tubes were incubated in the dark at 4°C, for 20 minutes. The cells were then washed in 2 mL of phosphate-buffered saline (PBS). Finally, 500 μL of PBS was added into tubes and cell concentrations were assessed by flow cytometry. The lymphocyte subset was determined using forward and side scatter (FSC/SSC) density plots. $CD3^+$ T cells and SSC were used for gating; $CD3^+$ T cells were used for the analysis of $CD4^+/CD25^+$ expression, i.e., the ratio of $CD4^+/CD25^+$ T to $CD3^+$ T cells was calculated.

Body temperature and infection

Nasopharyngeal and rectal temperatures were measured before surgery and at 2 and 4 hours after surgery. At 1 week after surgery, patients were examined for evidence of any incisional and deep organ infection. External validation of the evidence of surgical site infection (SSI) required observation of signs and symptoms of infection, including pain, tenderness, local swelling, redness or heat, and purulent drainage with confirmed causative microorganism according to the definition of SSI recommended by the Centers for Disease Control and Prevention (CDC) [25] and as previously described [26].

Statistical analysis

Continuous variables are presented as the mean ± standard deviation (SD) and categorical variables as counts and percentages. One-way analysis of variance (ANOVA) was performed to detect differences in continuous variables between the groups, whereas Chi-square test was used for evaluation of categorical variables. In order to investigate the overall effect of the time, group and time-group interaction, a two-way repeated measures ANOVA was performed. If the effect of time, group, or time-group interaction was significant, a *post hoc* test using the Bonferroni correction method was performed to control for the overall Type I error rate in multiple comparisons. The generalized linear model for group comparison and repeated measures ANOVA for time comparison were used as *post hoc* tests. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corporation, Armonk, New York, USA). Two-tailed $p < 0.05$ was set as statistically significant.

Power calculation

Since the aim of our study was to compare body temperature, immune function and wound infection rate, we selected these significant factors to perform the power calculation. For infection as a primary outcome, a sample size of 100 achieves 55% power to detect an effect size (W) of 0.255 using a Chi-square test with 3 degrees of freedom and a significance level (alpha) of 0.05. For body temperature as a primary outcome (nasopharyngeal temperature as reference), a repeated measures design with one "between" factor and one "within" factor has four groups with 25 subjects each or 100 subjects who are each measured three times. This achieves 5% power-to-test group effect and 100% power-to-test time effect with a 5% significance level and achieves 100% power-to-test interaction effect with 5% significance level. For immune function as a primary outcome (TGF-β as reference), a repeated measures design with one "between" factor and one "within" factor has four groups with 25 subjects each for a total of 100 subjects. Each subject is measured three times. This design achieves 86% power-to-test group effect and 100% power-to-test time effect with a 5% significance level and achieves 100% power-to-test interaction effect with a 5% significance level (Table 1).

RESULTS

Baseline patient characteristics

A total of 100 patients were included in the study and randomly assigned to 1 of 4 groups with 25 patients in each group.

The mean patient age was 74.99 years. No between-group differences in the baseline demographic and clinical characteristics and intraoperative fluid administration were observed (all $p > 0.05$; Table 2).

Comparisons of body temperature

Analysis of nasopharyngeal temperature showed significant differences by time, group, and time-group interaction (time effect, $p < 0.001$; group effect, $p < 0.001$; time-group interaction; $p = 0.002$). After Bonferroni correction in *post hoc* testing, the mean body temperature was significantly lower in Group D compared to Group A at 2 and 4 hours after surgery (both $p \leq 0.001$); in Group D compared with Group B at 4 hours after surgery ($p = 0.011$); in Group D compared with Group C at 2 and 4 hours after surgery [both $p \leq 0.041$] (Table 3).

For time effect, the mean nasopharyngeal temperature in all four groups was significantly lower at 2 hours after surgery compared to the preoperative values (all $p \leq 0.008$). In Group D, the mean body temperature was significantly lower at 4 hours after surgery compared to the preoperative value ($p < 0.001$).

The analysis of rectal temperature showed significant differences by group and time-group interaction (time effect, $p = 0.218$; group effect, $p < 0.014$; time-group interaction, $p = 0.030$). For group effects at each time point, the mean rectal temperature was significantly lower at 4 hours after surgery in Group D compared to Group A and C (both $p \leq 0.017$). No significant differences were found in time effect (Table 3).

Comparisons of TGF-β, IL-10, $CD3^+$ T cell, and $CD4^+/CD25^+$ Treg levels

Comparisons of TGF-β, IL-10, $CD3^+$ T cell, and $CD4^+/CD25^+$ Treg levels are shown in Table 4. For TGF-β, significant differences by time, group, and time-group interaction were found (time effect, $p < 0.001$; group effect, $p = 0.031$; time-group interaction, $p < 0.001$).

Post hoc testing with Bonferroni correction showed that the mean TGF-β level was significantly higher in Group A and B at 2 and 4 hours after the surgery compared with the preoperative TGF-β levels (all $p < 0.001$); was significantly higher in Group A and B at 4 hours compared to 2 hours after the surgery (both $p \leq 0.010$); was significantly higher in Group C and D at 4 hours after the surgery compared to the preoperative values (both $p \leq 0.031$). Significant differences in the mean levels of TGF-β were found in Group C compared to Group A and B at 2 and 4 hours after surgery (all $p \leq 0.015$).

The results for IL-10 showed no significant differences between the groups in any of the analyses (all $p > 0.05$).

The overall results for $CD3^+$ T cells indicated a significant

TABLE 1. Power analysis

Indicator	Group	Parameters							Power
		Mean	SD	Effect size	β/α	Total sample size	Number of measurements	Corr among rep measures[#]	
Temperature	A	36.56	0.56	0.454	4	100	3	0.9	0.943
	B	36.40							
	C	36.45							
	D	35.90							
CD3	A	52.88	12.5	0.109	4	100	3	0.9	0.319
	B	56.62							
	C	55.38							
	D	54.47							
Treg	A	9.07	1.33	0.312	4	100	3	0.9	0.778
	B	9.13							
	C	8.11							
	D	9.00							
TGF-β	A	104.61	14.40	0.406	4	100	3	0.9	0.905
	B	101.97							
	C	89.27							
	D	96.90							
IL-10	A	186.42	41.20	0.200	4	100	3	0.9	0.531
	B	190.18							
	C	168.37							
	D	182.23							

[#]Corr among rep measures: Correlation among repeated measures. One hundred patients scheduled for open surgery for gastric cancer were assigned to one of the four groups. Group A, both upper limbs, the lower 1/3 of lower limbs, and the head and neck were wrapped with a cotton pad; Group B, an insulation blanket was placed under the patient and heated to 39°C; Group C, infusion fluids, and blood if a transfusion was necessary, were heated to 37°C, peritoneal irrigation fluid was heated to 37°C, and an insulation blanket under the patient was heated to 39°C; Group D, infusion fluids, and blood if a transfusion was necessary, were heated to 37°C, peritoneal irrigation fluid was heated to 37°C, but a warmed insulation blanked was not used; CD3: Cluster of differentiation 3; Treg: Regulatory T cells; TGF-β: Transforming growth factor beta; IL-10: Interleukin 10.

difference in time effect (p = 0.001), but not in group effect or time-group interaction (both p > 0.05). *Post hoc* testing with Bonferroni correction showed no significant differences between any of the time points (all p > 0.05).

For $CD4^+/CD25^+$ Treg cells, the overall results showed significant differences in time effect (p < 0.001) and group effect (p = 0.023), but not in time-group interaction (both p > 0.05). *Post hoc* testing with Bonferroni correction showed that the mean $CD4^+/CD25^+$ Treg count was significantly higher in Group A at 2 and 4 hours after surgery compared to the preoperative values (both $p \leq 0.005$).

No significant differences were found in wound infection rates between the four groups (Table 4).

DISCUSSION

This study investigated the effectiveness of four different methods for controlling body temperature, aimed to prevent perioperative hypothermia, control immune function and reduce the rate of postoperative wound infections in patients undergoing open surgery for gastric cancer. The lowest temperature change (i.e., close to normal temperature) and cytokine response after surgery were observed in Group C, in which infusion fluids and transfused blood (if needed) were heated to 37°C, peritoneal irrigation fluid was heated to 37°C, and an insulation blanket was heated to 39°C and placed under the patient. The wound infection rate, however, was not significantly different between the four groups.

TABLE 2. Baseline and intraoperative data by group

Baseline and intraoperative data	n=25				*p*
	Group A	Group B	Group C	Group D	
Baseline characteristics					
Gender					0.847
Male n (%)	1 3 (52)	12 (48)	12 (48)	15 (60)	
Female n (%)	12 (48)	13 (52)	13 (52)	10 (40)	
Age (year)	72.88±8.23	76.56±5.55	75.16±7.63	75.36±7.32	0.344
Height (cm)	168.68±10.95	165.88±13.05	165.56±13.3	164.48±10.38	0.644
Weight (kg)	60.03±10.01	59.07±9.45	64.01±9.31	64.38±11.24	0.146
Preoperative systolic blood pressure	119.28±12.34	118.92±14.68	117±9.74	120.56±15.17	0.816
Preoperative diastolic blood pressure	71.48±8.47	72.76±7.48	74.64±8.36	74.64±9.51	0.481
Preoperative heart rate (beats/minute)	75.28±11.58	78.96±7.87	75.4±8.6	79.6±9.51	0.232
Urine volume	482.8±309.33	456±237.73	492±286.95	526.8±257.69	0.837
Intraoperative data					
Intraoperative irrigation (mL)	2726.8±1698.12	2366±1532	2514±1311.13	3172±1169.32	0.225
Intraoperative blood loss (mL)	413.6±776.01	317±233.01	421.6±425.49	344.8±227.76	0.827
Intraoperative fluid transfused (mL)	2184±1205.35	2360±852.45	1984±832.76	2268±605.17	0.500

One hundred patients scheduled for open surgery for gastric cancer were assigned to one of the four groups. Group A, both upper limbs, the lower 1/3 of lower limbs, and the head and neck were wrapped with a cotton pad; Group B, an insulation blanket was placed under the patient and heated to 39°C; Group C, infusion fluids, and blood if a transfusion was necessary, were heated to 37°C, peritoneal irrigation fluid was heated to 37°C, and an insulation blanket under the patient was heated to 39°C; Group D, infusion fluids, and blood if a transfusion was necessary, were heated to 37°C, peritoneal irrigation fluid was heated to 37°C, but a warmed insulation blanked was not used.

TABLE 3. Body temperatures of the four groups (°C)

Body temperatures	n = 25				*p* value for time effect	*p* value for group effect	*p* value for time-group interaction
	Group A	Group B	Group C	Group D			
Nasopharyngeal temperature					<0.001	<0.001	0.001
Preoperative	36.67 ± 0.27	36.5 ± 0.31	36.55 ± 0.29	36.56 ± 0.28			
2 hours after surgery	36.49 ± 0.33[a]	36.23 ± 0.37[a]	36.37 ± 0.39[a]	36.09 ± 0.34*‡[a]			
4 hours after surgery	36.56 ± 0.54	36.4 ± 0.62	36.45 ± 0.52	35.9 ± 0.55*†‡[a]			
Rectal temperature					0.218	0.014	0.030
Preoperative	36.67 ± 0.27	36.5 ± 0.31	36.55 ± 0.29	36.56 ± 0.28			
2 hours after surgery	36.69 ± 0.38	36.55 ± 0.4	36.72 ± 0.35	36.49 ± 0.46			
4 hours after surgery	36.82 ± 0.47	36.65 ± 0.63	36.81 ± 0.55	36.34 ± 0.5*‡			

One hundred patients scheduled for open surgery for gastric cancer were assigned to one of the four groups. Group A, both upper limbs, the lower 1/3 of lower limbs, and the head and neck were wrapped with a cotton pad. Group B, an insulation blanket was placed under the patient and heated to 39°C. Group C, infusion fluids, and blood if a transfusion was necessary, were heated to 37°C, peritoneal irrigation fluid was heated to 37°C, and an insulation blanket under the patient was heated to 39°C. Group D, infusion fluids, and blood if a transfusion was necessary, were heated to 37°C, peritoneal irrigation fluid was heated to 37°C, but a warmed insulation blanked was not used. *p< 0.05, significantly different from Group A; †p< 0.05, significantly different from Group B; ‡p < 0.05, significantly different from Group C; [a]p< 0.05, significantly different from preoperative value.

TABLE 4. Cytokine levels and CD T cell counts of the four groups

Cytokine levels and CD T cell counts	n=25				*p* value for time effect	*p* value for group effect	*p* value for time-group interaction
	Group A	Group B	Group C	Group D			
TGF-β level					<0.001	0.031	<0.001
Preoperative	79.82±11.55	80.92±12.34	83.23±11.87	82.69±13.95			
2 hours after surgery	100.98±13.36[a]	98.55±12.94[a]	86.71±13.36*,†	92.22±14.21			
4 hours after surgery	104.61±13.87[a, b]	101.97±14.63[a,b]	89.27±12.29*,†, [a]	96.9±16.61[a]			
IL-10 level					0.170	0.183	0.992
Preoperative	173.5±51.73	169.6±58.22	160.51±42.65	173.52±59.84			
2 hours after surgery	179.11±47.57	187.58±66.26	166.71±45.44	180.85±52.69			
4 hours after surgery	186.42±37.23	190.18±46.55	168.37±31.03	182.23±47.65			
$CD3^+$count					0.001	0.424	1.000
Preoperative	59.1±10.13	61.03±8.99	61.08±9.82	59.72±8.72			
2 hours after surgery	54.24±10.57	57.05±9.48	56.85±10.18	55.84±8.02			
4 hours after surgery	52.88±14.58	56.62±10.71	55.38±13.37	54.47±10.7			
$CD4^+/CD25^+$Treg count					<0.001	0.023	0.528
Preoperative	7.73±1.68	8.29±1.72	7.96±1.34	8.06±1.41			
2 hours after surgery	9.3±1.24[a]	9.44±1.39	8.64±1.04	9.25±1.63			
4 hours after surgery	9.07±1.6[a]	9.13±1.14	8.11±1.15	9±1.39			
Infection						0.09	
No n (%)	17 (68)	19 (76)	24 (96)	20 (80)			
Yes n (%)	8 (32)	6 (24)	1 (4)	5 (20)			

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The normal human body temperature is around 37°C and, in the perioperative period, a body temperature lower than 36°C is considered as hypothermia [27]. Perioperative hypothermia is more common in patients undergoing major operations such as laparotomy and thoracotomy, and when the duration of surgery is more than 2–3 hours [28]. Factors that may affect (e.g., lower) the body temperature during surgery include, among others, the surgical process, anesthetics, and surgical environment [29]. A reduction in body temperature during surgery can cause postoperative discomfort in a form of chills and limb numbness, which may lead to restlessness during recovery from anesthesia [29]. More importantly, hypothermia may be detrimental to the circulatory and immune systems, as well as to coagulation. This can lead to an increased incidence of cardiovascular events after surgery [30], increased intraoperative blood loss [31], reduced excretion of drugs by the kidneys and subsequent delayed recovery from anesthesia, and can also result in an increased rate of postoperative complications, including wound infections [32]. Evidence also suggests that even mild perioperative hypothermia can adversely affect immune function in patients [11,13]. Finally, all these factors can negatively affect patient outcomes and increase the duration of hospital stay, possibly increasing the economic burden of the patient.

The mechanism underlying the influence of temperature variation on the immune function during the perioperative period is still poorly understood. Here we assumed that the degree to which the methods for temperature control alter the immune function, including changes in cytokine production by Treg cells, depends on how well they are able to maintain the patient's body temperature close to normal.

CD_3^+ T cells represent mature T lymphocytes which have a crucial role in cell-mediated immunity [32]. Treg cells represent a subpopulation of T cells that express CD4, CD25, and forkhead box P_3 ($FOXP_3$) biomarkers, and have a major role in the control of immune tolerance, thus preventing autoimmune diseases. Treg cells are also involved in the maintenance of homeostasis, tumor immune surveillance, and induction of transplantation tolerance among other important biological processes [33]. In addition to $FOXP_3$, which regulates the transcription of specific genes associated with the development of functional Treg cells, it was demonstrated that the suppressive function of $CD4^+CD25^+$ Tregs is mediated by their production of IL-10 and TGF-β1 [24,33-35]. The results of our study showed that the production of cytokines and $CD4^+/CD25^+$ Treg cells was differentially affected depending on the method used for body temperature control and in relation to the different time points of measurement. Moreover, the approach in which the largest number of warming methods was combined (Group C) led to the smallest variation in body temperature and the lowest cytokine response.

Nevertheless, this study has several limitations. First, the number of patients in each group was relatively small, and we did not include a negative control group (i.e. a group

without application of the measures for perioperative temperature control). However, we assume that the negative control group would have similar results as the group in which only cotton pads were used to wrap the limbs. Second, the number of outcome measures was relatively limited, the SSI were not quantified or graded, and no other postoperative complications were analyzed. While the primary purpose of the study was to examine the immune function of patients, the use of other measures of immunity such as acute phase reactants (e.g. C-reactive protein) could have provided additional insights. Moreover, we did not evaluate other peri and postoperative outcomes such as patient pain severity, restlessness, duration of time spent in the recovery room and cardiac events, though the number of patients was likely too small to identify any differences in cardiac events between the groups.

CONCLUSION

In conclusion, the use of infusion fluids and blood heated to 37°C, peritoneal irrigation fluid heated to 37°C, and an insulation blanket heated to 39°C and placed under the patient proved to be the most effective in maintaining the patient's body temperature close to normal, during open surgery for gastric cancer. This method was also associated with the lowest changes in cytokine levels, indicating that the immune system was affected less than with the other methods. Further study is warranted to investigate whether maintaining normothermia during surgery results in less stress to the immune system and improved clinical outcomes.

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DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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