

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Study of the effects of IgM-enriched polyvalent immunoglobulin in an
E. coli-induced porcine sepsis model

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porcine sepsis model

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1. INTRODUCTION

Sepsis is a life-threatening condition characterized by multi-organ dysfunction resulting from the body's inadequate response to infection. Hospital mortality due to this condition is a significant public health problem worldwide. Sepsis is one of the world's leading fatal diseases, affecting more than 50 million people annually. Its mortality rate ranges from 20% to 50%, even with early detection and treatment. The average length of hospital stay for sepsis is twice as long as for any other fatal disease. Furthermore, sepsis survivors face an increased risk of mortality or a decline in health-related quality of life, even after discharge from the hospital. It poses a significant public health challenge even in developed countries: in the European Union, approximately 700,000 to 800,000 deaths occur annually due to sepsis, while the cost of treatment runs into the billions. The situation in Hungary is similarly alarming. According to data from the National Public Health Center, the number of hospital-acquired sepsis cases is increasing year by year, particularly in intensive care units, and therefore sepsis contributes significantly to the global health burden of disease.

The pathophysiology of sepsis is complex: the initial hyperinflammation is paradoxically followed by hypoinflammation (immune paralysis), which is the primary cause of secondary infections and organ damage. Current therapeutic guidelines—such as the Surviving Sepsis Campaign (SSC) protocol—emphasize the initiation of early antibiotic therapy, volume resuscitation, and organ support therapies; however, these are not always sufficient to reduce mortality. This is where treatment with IgM-enriched polyvalent immunoglobulin (IgM-enriched IVIG) (Pentaglobin®) comes into play. Due to their high IgM content, they effectively neutralize endotoxin, enhance phagocytosis, inhibit the cytokine storm, and restore the balance of the immune system. Clinical trials of Pentaglobin treatment show promising results: according to meta-analyses, mortality is reduced by 20–30% in patients treated for sepsis, particularly in Gram-negative infections.

However, there are contradictions in the literature regarding the optimal dose of IgM-enriched polyvalent immunoglobulin, the timing of infusion, and its selective application, partly due to heterogeneous patient populations. The aim of this dissertation is to investigate the effect of IgM-enriched polyvalent immunoglobulin in an *Escherichia coli* (*E. coli*)-induced porcine sepsis model. Our results may contribute to the refinement of treatment protocols, thereby improving the prognosis of the disease.

2. AIMS OF THE STUDY

1. Our objective was to investigate hemodynamic, hemorheological, and microcirculatory changes in the E. coli-induced sepsis model in pigs previously developed by our research group.
2. We set out to develop a protocol that would allow for the investigation of Pentaglobin administered prophylactically and therapeutically.
3. According to the developed protocol, we intended to study the effects of IgM-enriched immunoglobulin on hemodynamic, hemorheological, and microcirculatory parameters.
4. Finally, our goal was to compare the effects of polyvalent immunoglobulin administered prophylactically and therapeutically, and to draw conclusions regarding the different administration methods.

3. MATERIALS AND METHODS

3.1. Experimental animals and protocol

The experiment was conducted in accordance with the European Union Directive (EU Directive 63/2010) and the Hungarian Animal Protection Act (Act XXVIII of 1998 on the Protection and Welfare of Animals), and with the approval of the Animal Welfare Committee of the University of Debrecen (permit registration numbers: 18/2023/DEMÁB).

We provided ventilation (15–20 air changes per hour) and heating (central and floor heating) in the animal housing areas, maintaining a temperature between 22 and 26 °C depending on the animals' body weight. We avoided extreme and sudden fluctuations in humidity. The animals received feed mixtures appropriate for their species, and drinking water was provided via an automatic drinking system.

The experimental animals were divided into four different treatment groups:

1. Control group: they received only the fluid replacement used in the other groups.
2. E. coli bacteremia group: this group received an E. coli suspension.
3. E. coli parallel Pentaglobin (PG) group: the animals received immunoglobulin enriched with IgM simultaneously with the E. coli suspension.
4. E. coli delayed PG group: administration of Pentaglobin was initiated 60 minutes after the administration of the E. coli suspension.

Our experiment included thirty healthy, 12-week-old female Topigs Norsvin pigs, which were randomly divided into four groups. The control group (n=6) had an average weight of 24.5 ± 2.9 kg and a length of 91.3 ± 2.9 cm. The E. coli bacteremia group (n=8) had an average weight of 20 ± 2.5 kg and a length of 88 ± 5.4 cm. The E. coli parallel PG group (n=8) had an average weight of 22.3 ± 3.6 kg and a length of 88.4 ± 4.5 cm. The E. coli delayed PG group (n=8) had an average weight of 20.3 ± 1.5 kg and a length of 84.8 ± 2.8 cm.

The experiments were conducted under anesthesia. Following premedication with azaperone (2 mg/kg intramuscularly; Stresnil, Elanco GmbH, Cuxhaven, Germany), induction was performed by intramuscular administration of 15 mg/kg ketamine (CP-Ketamine hydrochloride 10%) and 1 mg/kg xylazine (CP-Xylazine hydrochloride 2%). Maintenance of anesthesia was ensured by intravenous administration of 1 mg/kg xylazine and 10 mg/kg ketamine. Following induction, endotracheal intubation was performed, followed by pressure-

supported mechanical ventilation (Aeonmed Respiratory Ventilator VG70, Beijing Aeonmed Co., Ltd., Beijing, China). The goal of mechanical ventilation was to maintain an arterial partial pressure of oxygen at 100 ± 10 mmHg and a partial pressure of carbon dioxide at approximately 40 ± 5 mmHg. Following surgical exposure of the right femoral artery, a PiCCo arterial catheter (Getinge PiCCo Catheter 4F, 16 cm, Pulsion Medical System SE, Feldkirchen, Germany) was inserted into the artery, while a central venous catheter (Certofix Duo 7F, 20 cm, B. Braun Trading Ltd., Budapest, Hungary) was placed in the external jugular vein for blood sampling, hemodynamic measurements, and administration of treatment. The operating room temperature was 25 degrees Celsius. We placed a heating pad under the animals to maintain a core body temperature of 37 degrees Celsius. A suprapubic cystostomy catheter was used to measure hourly urine output.

As in clinical practice, we administered fluid replacement to correct the hypovolemic state observed in sepsis. In the control, *E. coli* bacteremia, and PG treatment groups, the pigs were administered a physiological saline solution (“Baxter” 0.9% sodium chloride, pH = 4.5–7, osmolality: 308 mOsm/L, Baxter Hungary Kft.) at a rate of 10 ml/kg/h. The control group received only fluid replacement, while the animals in the other three groups received an *E. coli* suspension. In the *E. coli* bacteremia, *E. coli* parallel PG, and *E. coli* delayed PG groups, sepsis was induced in the same manner in all experimental animals.

The *Escherichia coli* culture (2.5×10^5 /mL; ATCC 25922, Institute of Medical Microbiology, Clinical Center, University of Debrecen) was administered to the pigs via continuous intravenous infusion in physiological saline as follows: 2 mL in the first 30 minutes, followed by 4 mL in the next 30 minutes, and the remaining 32 mL of bacterial culture was administered over 120 minutes. In total, the animals received 9.5×10^6 *E. coli* over 180 minutes.

In the parallel PG group, the animals received immunoglobulin enriched with 0.75 g/kg body weight of IgM via a 20-minute bolus infusion concurrently with the *E. coli* treatment. In the delayed PG group, a bolus dose of 0.67 g/kg body weight of Pentaglobin was administered one hour after *E. coli* administration, followed by a continuous infusion at a rate of 0.02 g/kg/h for 240 minutes. The animals in the parallel and delayed PG groups received the same amount of PG per kilogram of body weight. The volume of the immunoglobulin infusion was subtracted from the volume of the maintenance saline solution, resulting in a total volume of 10 mL/kg/h.

We observed the animals in all four groups for 6 hours; no animals died during the observation period. At the end of the experiment, the pigs were euthanized by intravenous administration of sodium pentobarbital (90 mg/kg, Release, WDT-Wirtschaftsgenossenschaft deutscher Tierärzte eG, Garbsen, Germany). During the study, no other medications (including

antibiotic treatment) or therapeutic interventions were administered, with the exception of intravenous anesthesia, physiological saline, the *E. coli* suspension, immunoglobulin, and pressure-supported ventilation.

3.2. Hemodynamic measurements

Hemodynamic changes were measured using the PiCCO™ device (PULSION Medical Systems AG, Munich, Germany), which operates on the principles of transpulmonary thermodilution and pulse waveform analysis. Measurements were performed using cannulas placed in the left external jugular vein and the left femoral artery. During thermodilution measurements, 10 ml ($T=8\text{ }^{\circ}\text{C}$) of normal saline was injected into the central venous catheter three times every two hours. The monitor plotted a thermodilution curve based on the temperature change. Using the area under the curve, heart rate, and calculated aortic compliance, we were able to monitor cardiac output beat by beat. Among the hemodynamic parameters, we measured cardiac index [CI ($\text{mL}/\text{min}/\text{m}^2$)], global end-diastolic volume index [GEDVI (mL/m^2)], intrathoracic volume index [ITBVI (mL/m^2)], extravascular lung water index [EVLWI (mL/kg)], systemic vascular resistance index [SVRI ($\text{dyn}\cdot\text{s}\cdot\text{cm}^5\cdot\text{m}^2$)], stroke volume variability [SVV (%)], pulse pressure variability [PPV (%)], heart rate [HR ($1/\text{min}$)], and mean arterial pressure [MAP (mmHg)] were recorded. During the experiments, all conditions necessary for interpreting PPV/SVV were met, including sinus rhythm, low tidal volume during mechanical ventilation, and low PEEP.

3.3. Determination of blood gas, metabolite, and hematological parameters

During the experiment, blood samples were collected every two hours to assess the involvement of various organs. An EPOC analyzer (Siemens Healthineers AG, Erlangen, Germany) was used to determine the partial pressure of oxygen in arterial blood (expressed in mmHg). We also used the same analyzer to measure lactate (mmol/L) and serum creatinine levels ($\mu\text{mol}/\text{L}$). The $\text{PaO}_2/\text{FiO}_2$ ratio was calculated based on the fraction of inspired oxygen (FiO_2) and the measured partial pressure of oxygen.

We used a Sysmex K-4500 automated hematology analyzer (TOA Medical Electronics Co., Ltd., Kobe, Japan) to measure qualitative and quantitative hematological parameters. The following parameters were evaluated in the study: red blood cell count (RBC [T/L]), white blood cell count (WBC [G/L]), hemoglobin concentration (Hgb [g/dL]), hematocrit (Hct [%]),

mean corpuscular volume (MCV [fL]), mean corpuscular hemoglobin (MCH [pg]), mean corpuscular hemoglobin concentration (MCHC [g/dL]), and platelet count (Thr [G/L]).

3.4. Determination of red blood cell aggregation

Red blood cell aggregation index values were measured using a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Roetgen, Germany). This technique employs transilluminance photometry and requires approximately 20 μ L of whole blood. After separation using a controlled shear system (shear rate: 600 s^{-1}), light transmittance was measured for 5 or 10 seconds under static conditions (M values, shear rate: 0 s^{-1}) or at low shear rates (M1 values, shear rate: 3 s^{-1}). (96,97) Measurements were performed at room temperature (20–25 °C). Higher index values (M 5 s, M1 5 s, M 10 s, M1 10 s) indicate a greater degree of red blood cell aggregation.

3.5. Determination of whole blood and plasma viscosity

Whole blood viscosity (WBV) and plasma viscosity (PV) were determined at 37 °C using a DVNext cone-plate rheometer with a CPA-40Z cone (AMETEK Brookfield, Middleborough, Massachusetts, USA). This technique requires 500 μ l of whole blood or plasma. When determining blood viscosity, values corrected for a 40% hematocrit were calculated using the mathematical formula recommended by Mátrai et al.

3.6. Microcirculation measurements

Non-invasive, direct visualization of microcirculation can be achieved using video microscopy. Orthogonal polarization spectral (OPS) imaging uses polarized light and detects the depolarized light scattered by moving erythrocytes. Side-dark-field (SDF) imaging uses an LED ring surrounding a central microscope to minimize surface reflections and improve signal quality. Incident dark-field (IDF) imaging, a refinement of SDF, provides centrally focused illumination, enabling higher resolution and contrast.

Video-microscopic examination of microcirculation was performed every two hours according to the experimental protocol. Using the CytoCam-IDF device, we recorded image sequences of the skin of the neck and ear, as well as the sublingual mucosa. Microvascular structures were visualized based on the presence of hemoglobin-containing erythrocytes; consequently, only blood vessels containing moving red blood cells were detectable. The device

illuminates the tissue with green light at a wavelength of 540 nm. The fluorescent light is absorbed by the erythrocytes and reflected by the surrounding tissues; the reflected signal is then captured by the CytoCam-IDF's 14-megapixel camera and converted into digital images.

We analyzed the videos using automated, validated analysis software (CytoCamTools V3 Bedside Manager, Braedius Medical, Huizen, Netherlands), which provides consistent microvascular parameters, including perfused vascular density (PVD)—the product of vascular network density and the proportion of perfused vessels—as well as the De Backer score. To visually characterize blood flow in the vessels, we used the MFI, or microvascular flow index, which is a dimensionless number.

3.7. Statistical analysis

Statistical analyses were performed using GraphPad Prism version 9.1.2 (GraphPad Software, San Diego, CA, USA). Data are presented as mean $\pm$ standard deviation (S.D.). The normality of the data distribution was tested using the Kolmogorov–Smirnov test, and accordingly, differences within groups were compared using repeated-measures ANOVA, followed by Dunnett's multiple comparison test or the Friedman test. Differences between groups were compared using an independent samples t-test/Mann–Whitney U test or one-way ANOVA, followed by Tukey's multiple comparison test/Kruskal–Wallis test. The significance level was set at $p < 0.05$.

4. RESULTS

4.1. Hemodynamic parameters

The PaO₂/FiO₂ ratio showed a decreasing trend in all experimental groups during the study. The most marked decrease was observed in the E. coli delayed PG group. Mean arterial pressure (MAP) decreased gradually starting six hours after the administration of the bacterial suspension only in the E. coli bacteremia group and the E. coli parallel Pentaglobin group. In the other groups (control and the E. coli delayed PG group), MAP remained relatively stable throughout the experiment. A slight decrease in platelet count was observed in all groups receiving the E. coli suspension, but not in the control group. A gradual increase in serum creatinine levels was observed in the experimental groups, but this did not occur in the control animals. During the experiment, a slight increase in serum lactate levels was observed in all groups.

4.1.1. Validation of the model in the control and untreated E. coli bacteremia groups

In the control animals, mean arterial pressure remained stable, whereas a decrease was observed in the E. coli bacteremia group (at 360 minutes: $p=0.0177$ vs. baseline; $p=0.0153$ vs. control). A gradual increase in heart rate was observed in both groups, which was more pronounced in the E. coli bacteremia group. The cardiac index rose slightly after an initial decrease at 120 minutes and was significant in the E. coli bacteremia group after 240 minutes ($p=0.0286$ vs. control). Systemic vascular resistance normalized in the control group after a transient increase, while a continuous decrease was observed in the E. coli bacteremia group (120 minutes: $p=0.033$; 240 minutes: $p=0.046$; 360 minutes: $p=0.006$).

No significant difference was observed between the control and E. coli bacteremia groups regarding GEDVI and ITBVI. For EVLWI, higher values were measured in the E. coli bacteremia group starting at 240 minutes compared to the control group. For SVV and PPV, we observed higher values in animals with untreated bacteremia compared to the control group. For SVV, we measured significantly higher values at 240 minutes compared to the control group ($p = 0.024$).

4.1.2. Comparison of hemodynamic changes between the E. coli bacteremia group and animals treated with Pentaglobin

In the groups treated with Pentaglobin (PG), we consistently observed higher MAP values compared to the untreated *E. coli* bacteremia group, a difference that proved significant in the delayed PG group for *E. coli* after 360 minutes ($p = 0.0058$ compared to the untreated bacteremia group). In the *E. coli* parallel PG group, a plateau in MAP values was observed after 60–180 minutes, followed by a decrease. Similarly, in the *E. coli* delayed PG group, a plateau in MAP values was observed after 120–240 minutes. An increase in heart rate was also observed in animals receiving PG treatment, but the values were lower compared to those in the *E. coli* bacteremia group. Furthermore, lower values were observed in the delayed PG treatment group compared to the parallel PG group. In the PG-treated groups, we obtained higher CI values after the start of immunoglobulin administration than in the *E. coli* bacteremia group. The increase in CI began after 60 minutes in the *E. coli* parallel PG group (not shown in the figure) and after 120 minutes in the *E. coli* delayed PG group. In the *E. coli* parallel PG group, significant differences were observed after 360 minutes compared to untreated bacteremic animals ($p = 0.0243$). In the SVRI values of treated animals, a decrease was observed following a transient increase (*E. coli* parallel PG at 60 minutes, *E. coli* delayed PG at 120 minutes), but these values remained higher throughout the study period compared to untreated bacteremic animals. The delayed PG group of *E. coli* showed significantly higher values after 360 minutes ($p = 0.0483$) compared to untreated animals with bacteremia.

The GEDVI and ITBVI scores were higher in both treated groups than in the bacteremia group. EVLWI values increased in both the PG-treated and untreated bacteremia groups, but higher values were measured in the untreated bacteremia group. In treated animals (*E. coli* parallel PG and *E. coli* delayed PG), SVV and PPV values were consistently lower than those in untreated bacteremia animals. In the *E. coli* parallel PG group, SVV values were significantly lower at both 120 minutes ($p = 0.0071$) and 240 minutes ($p = 0.0061$) compared to the *E. coli* bacteremia group. In the *E. coli* delayed PG group, a significant difference in SVV was observed only after 240 minutes ($p = 0.025$) compared to the *E. coli* bacteremia group. The PPV was significantly higher in the untreated bacteremia group than in both PG-treated groups from 120 minutes until the end of the study (*E. coli* parallel PG at 120 minutes: $p = 0.009$; 240 minutes: $p = 0.02$; 360 minutes: $p = 0.02$; *E. coli* delayed PG at 120 min: $p = 0.006$; 240 min: $p = 0.03$; 360 min: $p = 0.019$).

4.2.Hematological parameters

No significant changes in hematological parameters were observed in the control group during the study. In both the *E. coli* bacteremia group and the treated groups, the white blood cell count initially decreased and then began to rise. In the treated groups, a more pronounced increase occurred after 360 minutes (*E. coli* parallel PG 360 min: $p=0.0014$ vs. baseline; *E. coli* delayed PG 240 min: $p=0.0016$ vs. control, $p=0.0076$ vs. baseline). Red blood cell count, hemoglobin, and hematocrit showed a similar trend. We observed a slight increase in the *E. coli* bacteremic group, while the *E. coli* parallel PG and *E. coli* delayed PG groups showed a decreasing trend. Platelet count decreased in all groups; however, in the *E. coli* delayed PG group, this decrease was significant compared to baseline ($p=0.0443$ vs. 120 minutes, $p=0.0026$ vs. 240 minutes, $p=0.0051$ vs. 360 minutes).

4.3.Red blood cell aggregation

Red blood cell aggregation showed significant differences between the groups. Aggregation—defined as the reversible binding of cells under low shear rates or stasis—increased in all groups, but to a lesser extent in the treated groups (*E. coli* parallel PG, *E. coli* delayed PG). In the *E. coli* bacteremic group, larger aggregates and higher shear forces were required for disaggregation, whereas such changes were not observed in the treated groups. In the *E. coli* bacteremic group, we observed significant differences in several measured parameters compared to baseline values, as well as to the values of the control and treated groups. The most significant difference in stasis was observed at 10 seconds of aggregation. In the *E. coli* bacteremic group, the aggregation index values measured at 240 and 360 minutes were significantly higher than those in the control, *E. coli* parallel PG, and *E. coli* delayed PG groups ($p<0.0001$). Starting at 120 minutes after sepsis induction, these values also changed significantly compared to baseline ($p=0.0191$ vs. 120 minutes; $p=0.0003$ vs. 240 minutes; $p=0.0015$ vs. 360 minutes).

4.4.Whole blood and plasma viscosity

We measured whole blood viscosity at various shear rates. At low shear rates, both the *E. coli* bacteremic and treated groups (*E. coli* parallel PG and *E. coli* delayed PG) exhibited lower viscosity than the control animals after 6 hours. At higher shear rates, the control and *E. coli* delayed PG groups showed similar values, while the other two groups showed higher values.

The viscosity values measured at a shear rate gradient of 200 s^{-1} were nearly identical at all time points.

Plasma viscosity was lower in the *E. coli* bacteremia and *E. coli* parallel PG groups than in the other groups after 6 hours. The ratio of hematocrit to whole blood viscosity gradually increased in the *E. coli* bacteremia group, while in the treated groups (*E. coli* parallel PG and *E. coli* delayed PG) it normalized after 6 hours. Using the Mátrai formula (with viscosity corrected for 40% hematocrit), we observed a slight increase in the treated groups—more pronounced in the *E. coli* parallel PG group—yet after 6 hours, the results of both treatment groups correlated with the values of the control group.

4.5. Microcirculatory parameters

During the analysis of microcirculatory images, we observed a significant deterioration in the *E. coli* bacteremia group. Blood flow in the vessels slowed considerably, stasis developed in numerous capillaries, and we also observed areas of edema. In many cases, blood flow ceased entirely, and large red blood cell aggregates formed in the capillaries. The flow pattern was heterogeneous, with hyperdynamic flow in some capillaries and complete occlusion in others, reflecting the classic nature of sepsis-induced microcirculatory dysfunction.

In contrast, the groups treated with Pentaglobin (*E. coli* parallel PG and *E. coli* delayed PG) showed improvement. Both treatment regimens resulted in homogeneous circulation, similar to the flow pattern seen in the control images, significantly reducing stasis and the number of aggregates.

The numerical data obtained following offline analysis of the videomicroscopic recordings made during the study also reflect this. During the microcirculatory examination of the neck skin, we observed significantly slowed blood flow and frequent capillary stasis in the bacteremia group. The MFI (microvascular flow index) decreased significantly after 120 minutes before returning to baseline. De Backer scores and capillary density decreased in the *E. coli* bacteremia and *E. coli* parallel PG groups, while values in the *E. coli* delayed PG group remained similar to those in the control group.

Flow was frequently absent in the ear skin, with marked stasis, aggregates, and heterogeneous perfusion. MFI decreased in the bacteremia group at 120 and 240 minutes. De

Backer scores and capillary density were significantly higher in animals treated with Pentaglobin than in the *E. coli* bacteremia group.

Sublingual images showed similar abnormalities, with slowed capillary flow and aggregates. MFI differed from the control group in the *E. coli* bacteremic and *E. coli* parallel PG groups. De Backer scores and capillary density decreased in these groups, but Pentaglobin mitigated this decrease.

5. DISCUSSION

We monitored the development of sepsis based on the key parameters included in the SOFA (Sequential Organ Failure Assessment) score, which is recommended for describing the severity of sepsis. Since certain parameters (such as the GCS) could not be assessed during the studies underlying this thesis, we decided to monitor the following parameters during the experiment: the PaO₂/FiO₂ ratio to describe respiratory changes, mean arterial pressure (MAP values), platelet count, serum creatinine, and serum lactate. Changes in these parameters during the experiments supported the diagnosis of developing sepsis. In animals receiving the *E. coli* suspension, we observed a significant decrease in mean arterial pressure and systemic vascular resistance compared to control pigs, as well as increased stroke volume variability. These observations were consistent with previous similar reports. We found that IgM-enriched immunoglobulin treatment in this experimental setting contributed to higher mean arterial pressure and higher cardiac index values compared to untreated animals.

Consequently, in the Pentaglobin groups (whether administered concurrently with or following the induction of sepsis), we also observed a tendency toward an increase in the systemic vascular resistance index. It should be noted, however, that a statistically significant difference was observed only between animals with untreated sepsis and the group treated with Pentaglobin administered with a delay following the induction of sepsis at the 360-minute time point. Both pulse pressure variability (PPV) and stroke volume variability (SVV) parameters were significantly lower in animals treated with Pentaglobin. It is known from previous observations that a decrease in PPV may be an indicator of optimized fluid status. Since all experimental groups received the same volume of infusion, we hypothesize that the IgM-enriched immunoglobulin reduced fluid leakage into the interstitial space and/or increased systemic vascular resistance, thereby increasing circulating blood volume.

It is widely accepted that endothelial damage plays a crucial role in the development of sepsis. It has been demonstrated that the systemic release of TNF- α and IL- β cytokines induces increased expression of endothelial surface antigens, leading to leukocyte adhesion and microcirculatory disturbances. Previous experimental studies have already demonstrated the mitigating effect of IgM on microvascular perfusion insufficiency.

The pathophysiology of the beneficial effect of IgM-enriched polyvalent immunoglobulin is not yet fully understood. It has been shown that immunoglobulins contain high-affinity neutralizing antibodies that bind to IL-1, IL-6, and TNF- α , and these regulate

cytokine synthesis, leading to the stabilization of endothelial junctional connections. Furthermore, it has been suggested that immunoglobulins exert a neutralizing effect on microbial toxins and proinflammatory cytokines, have an anti-inflammatory effect, inhibit complement activation, and reduce microcirculatory dysfunction in sepsis. Based on experimental observations, intravenous immunoglobulins significantly reduce leukocyte adhesion and normalize capillary diffusion.

The aim of the study examining the concurrent administration of *E. coli* suspension and Pentaglobin was to evaluate the early anti-inflammatory effects of Pentaglobin (enhanced removal of endotoxins and reduced bacterial adhesion, invasion, and migration). The pathophysiological rationale for this was supported by previous findings from Barrett-Due and colleagues. Using a similar experimental model, they reported the binding of polyvalent immunoglobulins to lipopolysaccharide in the early stages of bacterial sepsis.

It should be noted that in an earlier experimental porcine sepsis model, in addition to the immunomodulatory effect, a delayed hemodynamic effect of IgM-enriched immunoglobulin was also observed in treated animals compared to untreated septic animals. Similar to our observations, they also documented a significant decrease in mean arterial pressure, an increase in heart rate, and a decrease in systemic vascular resistance. Although other detailed hemodynamic parameters are not available from this study, the percentage change in plasma volume remained consistently higher in the Pentaglobin group until the end of the experiments (240 minutes), which corresponds to the reduction in pulse pressure observed in the present study and may indicate improved microcirculation.

Pentaglobin treatment significantly reduced the increases in whole blood viscosity and red blood cell aggregation caused by experimental bacteremia, changes that underlie the microcirculatory disturbances characteristic of this model. The effect of delayed Pentaglobin treatment was more pronounced for both viscosity and aggregation parameters. This is presumably due to the treatment's superior pharmacokinetic profile and its immunomodulatory effect exerted in the early phase of sepsis. Pentaglobin treatment administered in parallel with sepsis induction also showed a positive effect, but to a lesser extent.

As a result of sepsis, we observed impairment of microcirculation in both the skin and mucosal capillary networks. These changes included reduced capillary perfusion, heterogeneous flow patterns, and a decrease in functional capillary density. Tissue oxygenation deteriorates as a combined effect of the release of inflammatory mediators, endothelial damage, increased capillary permeability, and microthrombus formation.

Based on our results, it can be concluded that Pentaglobin mitigates most of the damage associated with sepsis. In both dosing regimens, treatment was associated with improved microcirculatory parameters and partial preservation of tissue perfusion. These results suggest that Pentaglobin exerts a protective effect on microcirculation through immunomodulatory effects that reduce endothelial activation and limit further microvascular damage.

The acute-phase response that develops during sepsis increases fibrinogen production, a protein that plays an important role in the formation of red blood cell rouleaux and increased red blood cell aggregation. In addition, sepsis impairs red blood cell deformability and increases lipid peroxidation, thereby further impairing red blood cell aggregation. Reduced red blood cell deformability and oxidative damage can increase aggregation independently of plasma effects. In fulminant sepsis, increased aggregation and interactions between platelets and neutrophils can impair tissue perfusion by obstructing microvascular flow. A significant portion of rheological changes occurs in the early stages of sepsis and may serve as a potential diagnostic marker. Despite apparently adequate circulation, oxygen delivery is impaired, promoting the development of organ dysfunction.

The reduced deformability of red blood cells hinders their passage through narrow capillaries, contributing to capillary occlusion. In addition, stiffer red blood cells increase blood viscosity and resistance, which also impedes their passage through smaller capillaries. Increased blood viscosity and vascular resistance reduce perfusion and can cause heterogeneous hematocrit distribution. Stiffer red blood cells prolong the time required to pass through capillaries, reduce functional capillary density, and may promote capillary occlusion. These changes impair oxygen delivery, thereby exacerbating tissue hypoxia and potentially leading to organ failure.

Pentaglobin moderates the acute phase response by neutralizing endotoxins and cytokines, thereby inhibiting the rise in fibrinogen levels. By reducing inflammatory mediators, it can indirectly improve red blood cell membrane properties, thereby reducing oxidative damage, rigidity, and aggregation.

In a randomized, double-blind, placebo-controlled Phase II trial of sepsis, immunoglobulin enriched with IgM (Pentaglobin), administered at a dose of 5 ml/kg/day for 3 days, increased microvascular perfusion in the sublingual region. A 72-hour infusion of Pentaglobin increased perfused capillary density and the microvascular flow index, whereas these parameters deteriorated in the placebo group. However, the observed improvement occurred without a significant change in mean arterial pressure, and no correlation was found

between changes in microvascular perfusion and macrohemodynamic parameters. The study confirmed a decrease in IL-6 and IL-10 levels in the treated group, suggesting that Pentaglobin may alleviate sepsis-induced endothelial dysfunction and glycocalyx degradation. The mechanisms underlying these changes are not yet fully understood, and further research is needed to clarify them.

Clinical trials demonstrated a reduction in the severity of infection and in markers of organ dysfunction, thereby confirming the improvement in rheological properties. These effects were observed early on even at the standard dosage, promoting tissue oxygenation. A reduction in red blood cell aggregation, as well as a decrease in the number and size of aggregates at low shear rates, may reduce blood viscosity and improve homogeneous capillary perfusion.

One milliliter of Pentaglobin solution contains 50 mg of plasma protein, at least 95% of which is immunoglobulin: IgM (6 mg/mL), IgA (6 mg/mL), and IgG (38 mg/mL). Pentaglobin at a concentration of 1 mg/mL inhibits lymphocyte proliferation and cytokine release in vitro and in vivo in rats, which indirectly reduces fibrinogen-mediated red blood cell aggregation.

Research indicates that, when standard dilutions are used, opsonizing activity and reduced platelet aggregation can be observed. At higher doses, IgG-dominant IVIG analogs exhibit a weaker anti-inflammatory effect compared to IgM-enriched fractions. At higher concentrations, such as IVIG containing 10 times more IgG, a weaker effect was demonstrated, which may indirectly prove the efficacy of IgM. However, further targeted studies are needed to clarify the exact mechanism and the relationship between red blood cell aggregation and dosage.

IgM is a pentameric molecule that binds with high affinity to bacterial endotoxins (lipopolysaccharides), thereby promoting phagocytosis and clearance. Thus, it systemically reduces the extent of inflammation and the acute-phase response that elevates fibrinogen levels, thereby moderating the formation of red blood cell rouleaux.

Studies have demonstrated lower fibrinogen concentrations following administration of Pentaglobin. This is due to moderate platelet activation and a decrease in coagulation. Currently, there are no structural or affinity studies to confirm interactions between IgM and the D-domains of fibrinogen or other sites that promote rouleaux formation.

It is important to note some limitations of the study. The most significant limitation of the present study is its translational nature; the results of animal experiments cannot be directly applied to clinical practice. Sepsis was induced by intravenous administration of *E. coli*, which, while representing a specific Gram-negative form of fulminant sepsis, is not necessarily representative of other Gram-negative or Gram-positive forms of severe sepsis. Pre-treatment

with immunoglobulins is not realistic in clinical practice; this should be considered a “bench-to-bedside” limitation. Delayed Pentaglobin treatment was initiated at a specific time point in the experiments, which is also not the case in clinical practice. However, since we wanted to standardize our experiments, we ultimately decided to administer the immunoglobulin at time points that were not based on clinical symptoms. It is known that in the intravenous *E. coli* sepsis model, the clinical course is rapid; pathogens introduced into the bloodstream exert their most significant effect on the endothelium and, consequently, on the vascular system. The developing hypodynamic circulation is accompanied by elevated cytokine levels, and without therapeutic intervention, the outcome is fatal. In our study, we did not measure cytokine levels, nor did we investigate the precise mechanism of action of intravenously administered IgM-enriched immunoglobulins; these must be examined in the future. We believe that despite these limitations, the present study has contributed new information toward a better understanding of the pathophysiological processes occurring during *E. coli*-induced sepsis, as well as the hemodynamic, hemorheological, and microcirculatory changes.

6. KEY FINDINGS AND CONCLUSIONS

1. In the *E. coli*-induced porcine sepsis model previously developed by our research group, we described the hemodynamic, hemorheological, and microcirculatory changes that occur during sepsis. We found that, compared to control and baseline values, mean arterial pressure progressively decreases, systemic vascular resistance decreases, stroke volume variability increases, whole blood viscosity increases, and red blood cell aggregation intensifies.
2. Our developed protocol proved methodologically suitable for the hemodynamic, hemorheological, and microcirculatory assessment of different modes of Pentaglobin administration.
3. During parallel administration of Pentaglobin, the cardiac index and global end-diastolic volume index were higher, while stroke volume variability and red blood cell aggregation showed lower values. Pentaglobin treatment resulted in more homogeneous microvascular circulation, reducing stasis and the number of aggregates.
4. In the case of therapeutically administered, delayed Pentaglobin treatment, compared to concurrently administered immunoglobulin treatment, we observed higher systemic vascular resistance and mean arterial pressure values, a lower pulmonary water index, and a further reduction in stroke volume variability, as well as lower red blood cell aggregation values. Similar to the parallel treatment, a favorable microvascular circulation pattern was observed.

7. PUBLICATIONS



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Registry number: DEENK/233/2026.PL
Subject: PhD Publication List

Candidate: Balázs Ujhelyi

Doctoral School: Doctoral School of Neurosciences

List of publications related to the dissertation

1. **Ujhelyi, B.**, Mátrai, Á. A., Berhész, M., Molnár, L. P., Deák, Á., Tóth, Z., László, I., Németh, N., Fülesdi, B.: Hemodynamic Effect of IgM-Enriched Immunoglobulin in the Early Stage of E. coli-Induced Experimental Sepsis.
J Clin Med. 15 (4), 1-13, 2026.
DOI: <http://dx.doi.org/10.3390/jcm15041522>
IF: 2.9 (2024)
2. Mátrai, Á. A., **Ujhelyi, B.**, Varga, Á., Záhorszki, S., Adorján, D. M., Tóth, Z., Németh, N., Fülesdi, B., Deák, Á.: Hemorheological and microcirculatory effects of Pentaglobin therapy in an experimental model of fulminant sepsis.
Front. Physiol. 17, 1-12, 2026.
DOI: <http://dx.doi.org/10.3389/fphys.2026.1816549>
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List of other publications

3. Bekéné Debreceni, I., Krajcsir, B., Mátrai, Á. A., **Ujhelyi, B.**, Pócsi, M., Balla, G. J., Nagy, B. J., Deák, Á., Németh, N., Fülesdi, B., Kappelmayer, J.: IgM-enriched immunoglobulin treatment significantly improves coagulation dysbalance in an experimental animal model of fulminant sepsis.
Thromb. Res. 259, 1-8, 2026.
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