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Gastric Tonometry and Impedance Spectroscopy as a Guide to Resuscitation Therapy During Experimental Septic Shock in Pigs

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Abstract. *Background: This work investigates the potential value of combined splanchnic perfusion and tissue injury measurements as a guide to resuscitation therapy during an experimental model of septic shock in anesthetized pigs. Materials and Methods: An endotoxic shock model in 22 male pigs was developed by intravenous infusion of lipopolysaccharide. Three experimental groups were designed: a control group, a therapy group whose intervention was guided by haemodynamic variables, and a therapy group whose intervention was guided by splanchnic tissue indicators. The control group was allowed to progress into septic shock without intervention. The animals were subjected to resuscitation protocols with fluids and catecholamines depending on their responses, either to haemodynamic variables in one treatment group, or to measurements of pH_i combined with an ischemic injury classification given by gastric impedance spectroscopy in the other treatment group. Resuscitation protocols were designed to promote changes in haemodynamic responses as well as splanchnic perfusion. A Kaplan-Meier survival analysis was estimated in each group. Results: Survival in both treatment groups was significantly better than in the control group. There was no significant difference in the survival outcome between treatment groups. Conclusion: Splanchnic tissue indicators have the potential of being used as complementary tools for guiding the appropriate treatment of septic shock. The results justify the need for further studies in order to determine the best use of gastric tonometry and spectroscopic impedance information in clinical practice.*

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Splanchnic hypoperfusion plays a critical role in the development of shock and multiple organ failure (MOF) (1). Before any haemodynamic insufficiency is evident at the systemic level, splanchnic hypoperfusion can be present and may lead to gastrointestinal ischemia (2, 3). If ischemia lasts long enough, the intestinal mucosa degrades, losing its barrier function and allowing endotoxin and bacterial translocation (4). This mucosal disruption is the trigger of the decompensation process in shock (5), and the "motor of MOF" (6). Gastrointestinal ischemia occurs in more than 50% of intensive care patients and has been associated with 80% of deaths (7).

Several therapies with fluids, catecholamines (8-13) and nitric oxide synthase modulators (14, 15) focusing on avoiding or limiting the negative effects of ischemic injury of the gastrointestinal mucosa, have been developed and evaluated. However, the effectiveness of such therapies and their effect on splanchnic perfusion or patient outcome has been controversial. Measurement of intramucosal pH (pH_i) (13, 16-18) and gastric mucosal PCO_2 relative to arterial P_aCO_2 (PCO_2 gap) (19, 20) have been used as indicators of intestinal ischemia in order to prevent MOF and death in the critically ill. Both pH_i and PCO_2 gap measurements seem to be useful as early warning signs for inadequate perfusion (21, 22), yet do not estimate the degree of mucosal damage. After prolonged ischemia, it is difficult – if not impossible – to estimate the level of tissue injury (for instance, if the measured pH_i is already abnormal in admitted patients). This lack of discriminating information may explain the conflicting results obtained with pH_i-guided resuscitation (11-13) (hyper-resuscitation may be counterproductive after excessive mucosal damage). Furthermore, improved outcomes may depend on more specific splanchnic indicators in order to help guide therapies in the critically ill.

Impedance spectroscopy provides good information about tissue structure (23). Complex impedance spectroscopy

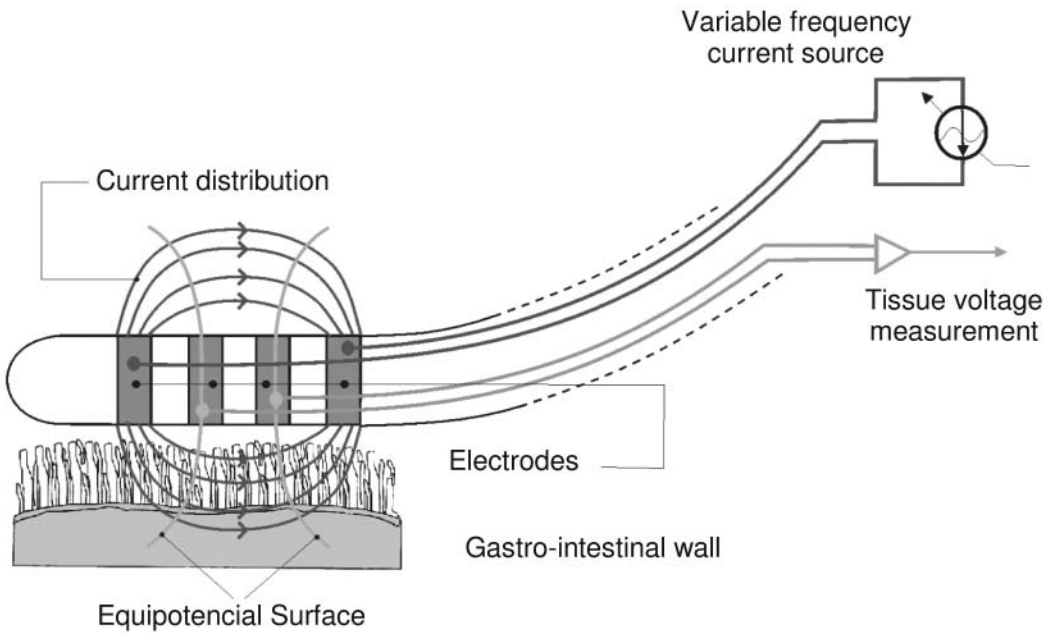

Figure 1. *Four-electrode system of tissue complex impedance measurement. The current source produces a constant sinusoidal excitation in the tissue through both external electrodes at pre-programmed frequencies. The two internal electrodes make complex voltage measurements.*

provides amplitude and phase information, allowing the separation of resistive and reactive tissue components. Electrical resistance reflects the conducting properties of electrolytes. But cell membranes also act as dielectrics, adding a reactive element to the homeostatic balance of the cell. Thus, overall impedance reflects the interaction of both resistance and reactance components within a complex tissue structure. Changes in extra- and intracellular volume (such as edema), membrane ion permeability, ion pump dysfunction and/or membrane disruption all directly affect the complex impedance of a given tissue at different frequencies.

Previous studies have proposed the use of impedance spectroscopy to measure changes in ischemia or tissue perfusion (24, 25) and to assess levels of tissue damage in different organs (26, 27). Our group has proposed the use of impedance spectroscopy as a tool for monitoring ischemic injury in the intestinal mucosa of the critically ill (28). In a previous study, haemodynamic variables and regional perfusion indicators such as pH_i and PCO_2 gap have been compared to gastric impedance spectroscopy measurements with the purpose of finding potential differences in survival outcome of patients undergoing elective cardiovascular surgery (29, 30). Data from that study strongly suggested that gastric impedance spectra might be used as a good predictor of patient outcome after cardiac surgery. An additional study in patients undergoing cardiovascular surgery evaluated spectral differences between patients exhibiting no evidence of gastric ischemia or other complications and patients who had developed ischemia and/or other complications (30). The parameters obtained for gastric tissue showed dramatic changes at different times and at different frequencies as a function of ischemia progression, and could furthermore be correlated with patient outcome.

The objective of this study was to investigate the potential value of combined measurements of pH_i and gastric impedance spectroscopy as modulators of resuscitation therapy in cases of shock. Two experimental therapy protocols were designed and compared: the first was guided by haemodynamic variables, the second, by splanchnic tissue measurements. Our goal was to compare the relative value of measurements from the oxygen supply side to those from the oxygen demand side for each subject.

Materials and Methods

Impedance spectroscopy. A prototype gastrointestinal spectroscopy system as previously described by Sacristán (31) was used. This system includes a four-electrode gastric catheter (32), as well as a PC as interface and platform for spectral processing. The spectrometer generates an excitation current of approximately 2 mA at 25 different frequencies within the range of 0.1 to 1000 KHz. This current is injected into the tissue through the external electrodes of the catheter (Figure 1). The internal electrodes measure the potential generated in the tissue by the excitation current. The complex impedance spectrum (amplitude and phase) was calculated in software at pre-programmed frequencies sweeping the range of interest, and was used to estimate an ischemic injury level of the gastric mucosa.

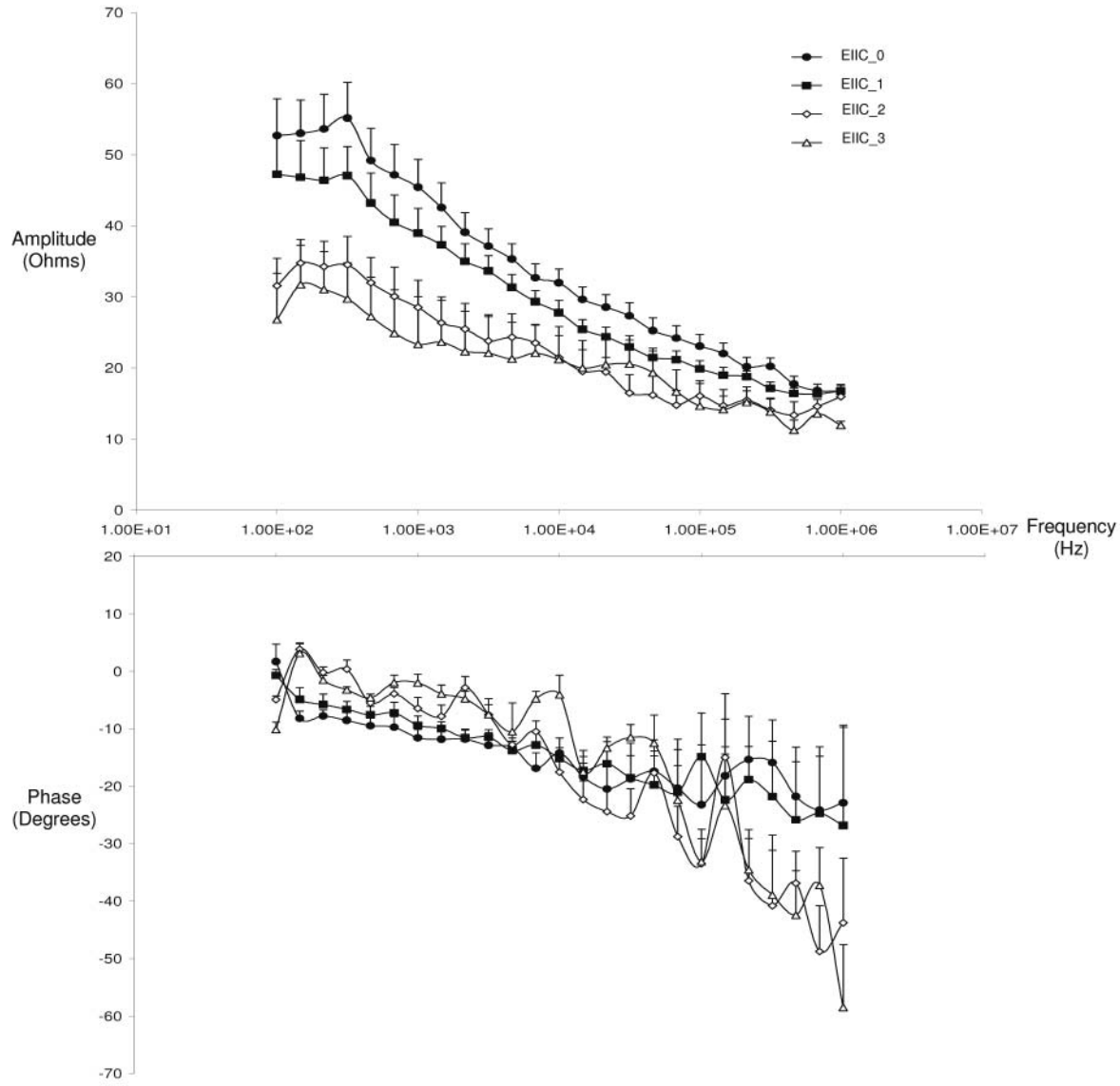

Figure 2. *Amplitude and phase spectra of the control group data, classified according to their corresponding EIIC. These data were used to calibrate a pattern recognition system of ischemic injury designed by a LVQ neural network.*

Surgical procedures and septic shock protocol. The Animal Experiments and Ethical Committee of the Institution approved all experimental protocols and the care of animals was carried out in accordance with the Declaration of Helsinki and IASP guidelines (33, 34). Twenty-two male pigs weighing 9 to 12 kg were sedated intramuscularly with azaperone (0.5 ml 20 kg^{-1}), anesthetized intravenously (*i.v.*) with sodium pentobarbital (17 mg kg^{-1}) and subsequently maintained as required via the same route. The animals were mechanically ventilated in order to maintain the end-tidal carbon dioxide concentration ($EtCO_2$) at approximately 40±5 mmHg at a tidal volume of 10 ml kg^{-1}. The oxygen/air proportion was adjusted to maintain the arterial oxygen saturation (S_aO_2) at approximately 96%±1. Polyethylene (PE160) catheters were placed in both the right femoral artery and the right femoral vein for continuous monitoring of the mean arterial blood pressure (MABP), to obtain samples and to infuse *i.v.* fluids. A pulmonary artery catheter was placed in the pulmonary artery to measure pulmonary artery wedge pressure (PAWP) and cardiac output (by thermodilution). An estimated cardiac index ($_eCI$) was calculated by dividing cardiac output by body weight as reported by Fink *et al.* (35) where an $_eCI=0.1$ corresponded roughly to 2.5 l min^{-1} m^{-2}. Peripheral vascular resistance (PVR) was calculated by dividing MABP by $_eCI$. In this study, the central venous pressure (CVP) remained close to zero and was ignored in the estimation of PVR. The animals were heparinized (200U kg^{-1}, *i.v.*) and after the course of one hour of stabilizing conditions, the experiment commenced at time zero (t=0).

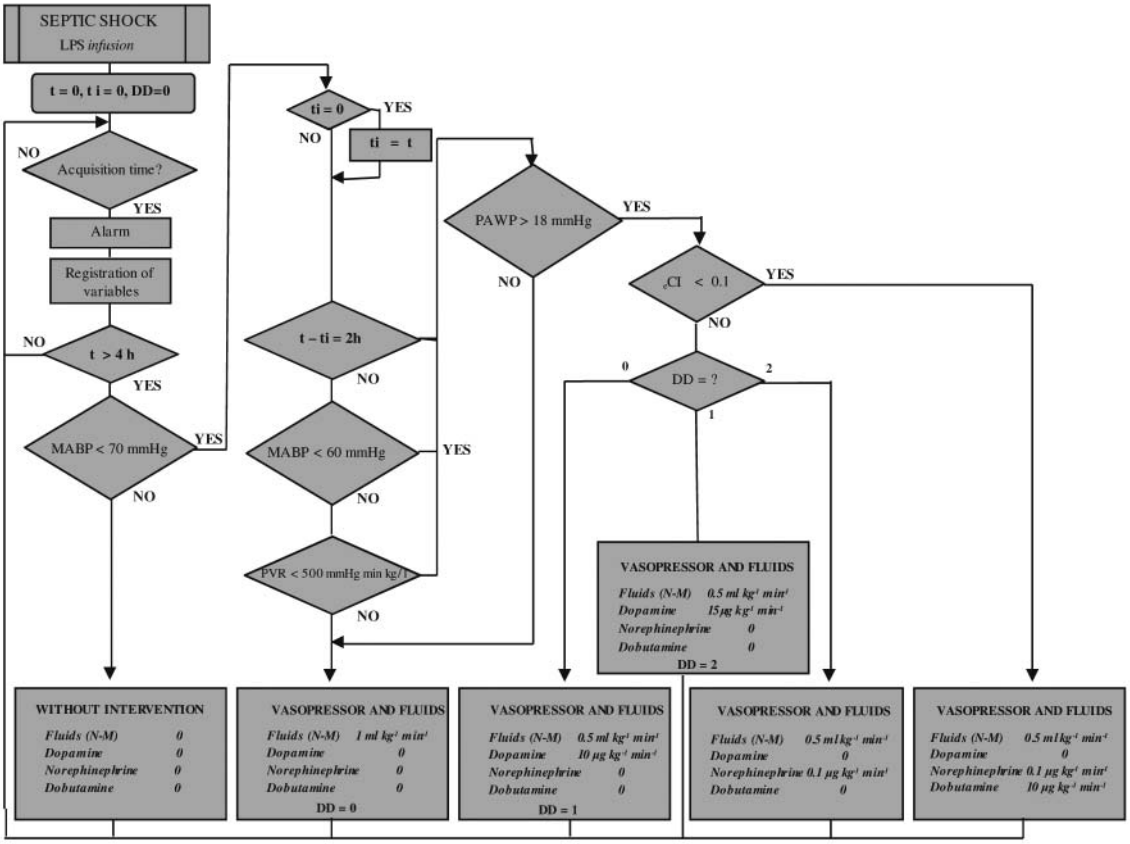

Figure 3. *Experimental protocol of resuscitation corresponding to group II: therapy-guided-by-haemodynamic-variables. LPS was infused at t=0 h and the septic shock model was allowed to progress for 4 h. Subsequently (t>4 h), a MABP<70 mmHg triggered the therapeutic intervention and enabled the timer (t_i). The initial intervention involved only fluids at a dose of 1 ml kg^{-1} min^{-1} during a maximum of 2 h (t-t_i=2 h). The variable DD (0 to 2) defines three sequential doses of catecholamines administered. A complete experimental protocol of resuscitation cycle was terminated every 30 minutes.*

In all animals, arterial and venous samples, as well as complex impedance spectra were obtained every 60 minutes beginning at t=0 until t=720 min (12 h). Gas analysis was obtained from arterial and venous samples using an automated blood analyzer (i-Stat, Abbott, Abbott Park, Illinois, USA). A balloon tonometer and Tonocap monitor (Datex-Ohmeda, Division Instrumentarium Corp., Finland) were used to obtain gastric intramural PCO_2 every hour. Arterial bicarbonate concentration and P_aCO_2 were used to calculate pH_i by a modification of the Henderson-Hasselbalch equation as proposed Fiddian-Green *et al.* (2). The PCO_2 gap was also obtained using the intramural PCO_2-P_aCO_2 difference, as proposed by Friedman *et al.* (36).

The porcine endotoxic shock model used in this study was adapted from a previous model described by Breslow *et al.* (37) consisting of an infusion of *Escherichia coli* lipopolysaccharide (LPS) for 120 minutes starting at t=0, at a dosage of 160 µg kg^{-1} per hour. As soon as the experiment concluded, euthanasia was performed by intravenous administration of KCl solution.

Experimental design. Three experimental groups were designed: group I (Control, n=6), group II (therapy guided by haemodynamic variables, n=8), and group III (therapy guided by splanchnic tissue indicators, n=8). The control group was evaluated before other treatment groups in order to obtain impedance spectroscopy data used to calibrate the pattern recognition system that estimated ischemic injury level (see section on pattern recognition system below). The animals in the intervention groups were randomly distributed.

In the control group, the septic shock model was allowed to progress without intervention, whereas in the treatment groups, the animals were subjected to experimental resuscitation protocols (described below) four hours after LPS infusion. All subjects were observed during a time-period of eight hours following the beginning of each intervention therapy, and thus, for a total of 12 hours for each experiment.

Classification and pattern recognition of ischemic injury. A pattern recognition classification system was developed using artificial neural networks and calibrated using impedance spectra as well as an integration of the pH_i change over time of the control group data. The system analyzes each complex impedance spectrum and produces an estimated ischemic injury classification (EIIC) with four classes, on a scale of 0 to 3, where 0 stands for a healthy tissue and 3 for a totally infarcted tissue. Figure 2 shows the amplitude and phase spectra of the control group data classified according to their corresponding EIIC. These data were used to calibrate a

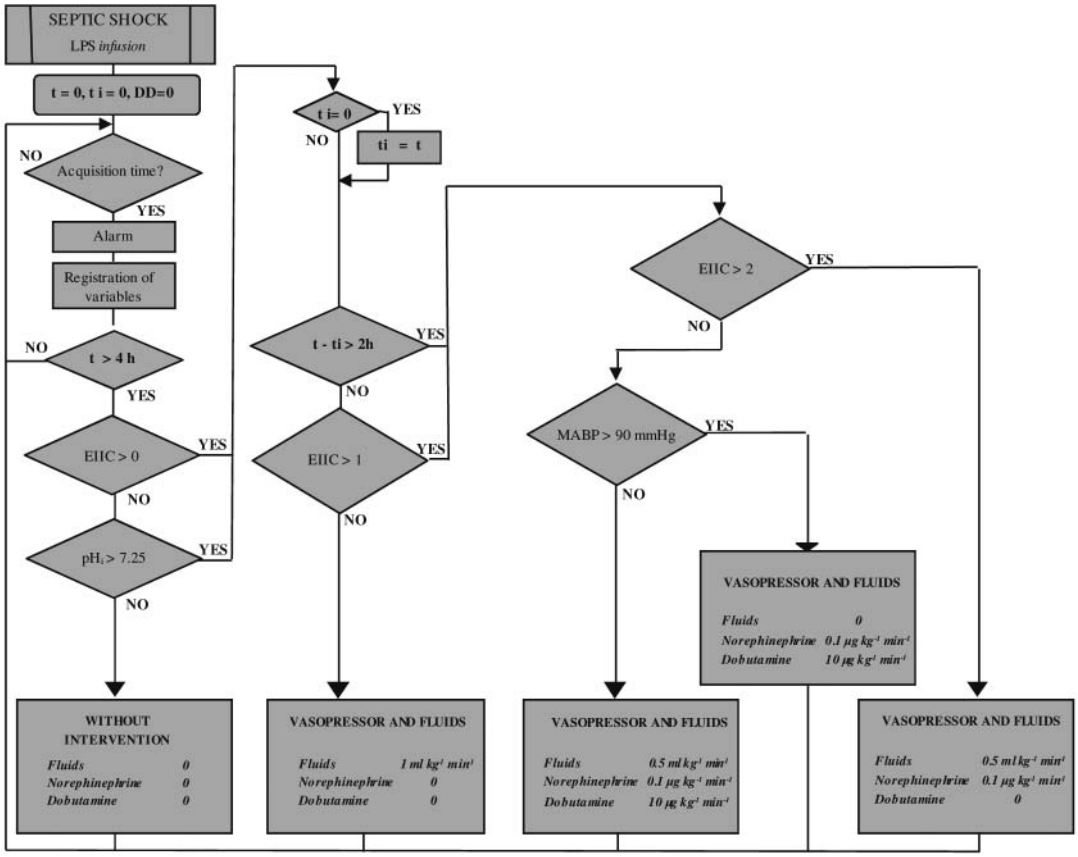

Figure 4. *Experimental protocol of resuscitation corresponding to group III: therapy-guided-by-splanchnic-tissue-indicators. LPS was infused at t=0 h and the septic shock model was allowed to progress for 4 h. Subsequently (t>4 h), an IIC>0 or pHi<7.25 triggered the therapeutic intervention, enabling the timer (t_i). If EIIC=1, the initial intervention involved only fluids at a dose of 1 ml kg^{-1} min^{-1} during a maximum of 2 h (t-t_i=2 h). A complete experimental protocol of resuscitation cycle was terminated every 30 minutes. See Methods section for details.*

pattern recognition system of ischemic injury designed by a learning vector quantization (LVQ) neural network. A detailed description of the algorithm used, its development and calibration has been presented by González *et al.* (38).

Experimental therapy protocols. Two experimental resuscitation protocols were designed for each treatment group and are presented as flowcharts in Figures 3 and 4.

'Therapy-guided-by-haemodynamic-variables' group (Group II): Measurements of MABP, PAWP, $_e$CI and PVR were used with focus on maintaining normal haemodynamic values. A MABP<70 mmHg triggered therapeutic intervention. The initial treatment therapy was aimed at raising the MABP to normal levels *via* fluid administration (Normosol-M DX 5%, Hospira, Inc., Lake Forest, IL., USA) at a dose of 1 ml kg^{-1} min^{-1} for a maximum of two hours). The administration of a combination of fluids (Normosol-M DX 5% at a dose of 0.5 ml kg^{-1} min^{-1}) with catecholamines was considered if either of the following conditions were present: a PAWP>18 mmHg (and the animal did not respond during the first two treatment hours with fluids), a MABP<60 mmHg, or a PVR<500 mmHg min kg l^{-1}). The combination of fluids and catecholamines was designed to maintain adequate MABP (39-42) and splanchnic blood flow (41, 43). Catecholamine treatment began with the administration of dopamine at increasing doses (DD=0: dopamine 10 μg kg^{-1} min^{-1}, DD=1: dopamine 15 μg kg^{-1} min^{-1}). If the animal did not respond to dopamine, norepinephrine (43-48) was used (DD=2) at 0.1 μg kg^{-1} min^{-1}. If the estimated cardiac index was clearly abnormal ($_e$CI<0.1), the immediate action was to administer a combination of dobutamine and norepinephrine at doses of 10 μg kg^{-1} min^{-1} and 0.1 μg kg^{-1} min^{-1} respectively. The main purpose of using this combination was to improve the $_e$CI by increasing the heart rate (45, 49-51).

'Therapy-guided-by-splanchnic-tissue-indicators' group (group III): Measurements of pH_i and EIIC were used to maintain normal pH_i values and an EIIC=0. A pH_i<7.25 or an EIIC>0 triggered the therapeutic intervention. This consisted initially on recovering the pHi or EIIC by fluid administration (Normosol-M DX 5% at a dose of 1 ml kg^{-1} min^{-1} during a maximum of 2 h). If the animal did not respond during the first 2 h of treatment, or its EIIC >1, a moderate gastrointestinal ischemic injury was assumed and a combination of dobutamine and norepinephrine was administered (10 μg kg^{-1} min^{-1} and 0.1 μg kg^{-1} min^{-1}, respectively) in order to improve the pH_i (12, 13, 52). If MABP remained below 90 mmHg, Normosol-M DX 5% was added at a dose of 0.5 ml kg^{-1} min^{-1}. If EIIC>2, an irreversible gastrointestinal ischemic injury was assumed; to prevent further

Table I. *Average survival time and total fluid load for each group. The average total fluid load in group III was less than in group II. A t-test for independent samples did not show statistical differences in the amount of fluids used.*

Group	Survival time (hours)	Total fluid load (ml)
I	6.79±3.09	-
II	9.18±1.83	1,236.87±661
III	9.56±2.03	1,083.12±418

Table II. *Values of time, PCO_2 gap, pH_i and EIIC at the beginning of the intervention for each treatment group. Values are shown as mean and standard deviation. A t-test for independent mean showed statistical differences for the EIIC estimated.*

Group	Time to intervention (hours)	PCO_2 gap (mmHg)	pH_i	EIIC (0-3)
II	5.56±1.98	45.11±19.64	6.68±0.59	2.3±0.44
III	4.18±0.25	33.96±8.95	7.05±0.07	1.0±0.70

damage by reperfusion or to control the spread of translocated bacteria, the splanchnic circulation was limited with the use of Normosol-M DX 5% at a dose of 0.5 ml kg^{-1} min^{-1} together with norepinephrine at a dose of 0.1 μg kg^{-1} min-1 (41, 43).

Statistical analysis. Differences in the time-course of MABP, PAWP, $_eCI$, PVR and PCO_2 gap were estimated using a two-way ANOVA test. All results are shown as mean±standard error of the mean of *n* experiments. Cumulative survival proportion and risk rate were estimated by a Kaplan-Meier and a logarithm range analysis. In the treatment groups, a *t*-test for independent samples was computed for values of time, PCO_2 gap, pH_i and EIIC at the start of the intervention. All statistical analyses were developed using STATISTICA, version 1997 (StatSoft Inc., Tulsa, OK, USA) The significance level was established at $p<0.05$.

Results

All animals in the control group perished before t=12 h. In group II, six animals perished and two animals survived. In group III, five animals perished and three animals survived. In both intervention groups the highest mortality was observed at around 8 h. The total fluid load used during the intervention protocols was lowest in group III compared to group II. Table I shows the average values of survival time and total fluid load for every group.

The time-course of MABP is shown in Figure 5. MABP decreased during the infusion of LPS. A partial recovery of the MABP in the control group was evident after 4 h and, as shown in Figure 6, was maintained by a high PVR. These changes correspond to the compensatory period of septic shock. In the control group, decompensation, which was manifested by a fall in MABP and PVR values, began approximately 7 to 8 h post LPS infusion. Resuscitation using fluids and catecholamines allowed the MABP to increase in both treatments groups, while decompensation ensued in the control group. This change became more evident when therapy was guided by splanchnic tissue indicators (Figure 5). Resuscitation also modified the pattern of changes of PVR shown in the control group (Figure 6). The continuous monitoring of the mean venous blood pressure showed no significant changes in any of the groups.

Furthermore, the $_eCI$ decreased after infusion of LPS in all groups (Figure 7). At t=4 h, the $_eCI$ was approximately 0.1. At t=6 h, the $_eCI$ recovery improved in group III when compared to group II. In the control group however, the $_eCI$ continued to decrease.

The PAWP exhibited a continuous increase beyond normal limits (PAWP>15 mmHg) in the three groups but was less evident in the treatment groups. Resuscitation groups showed a fall in PAWP at the end of the experiment, which was more evident when treatment was guided by haemodynamic variables (Figure 8). The time-course of the PCO_2 gap showed continuous increment in the three experimental groups. After the resuscitation protocols, the PCO_2 gap of group III improved more than in group II. Unfortunately, after 4 h into each experiment, the data collected from the PCO_2 gap were not sufficient to determine any differences among groups (Figure 9).

The EIIC increased in all groups; after resuscitation, the EIIC of group III remained below that of group II. Before t=8 h, both treatment groups presented EIIC values which were close to 3 (Figure 10).

A cumulative survival proportion estimated by the method of Kaplan-Meier limited products, using a test of logarithm range and assuming a χ^2 distribution, is shown in Figure 11. The risk rate was greater in the control group (approximately 2.4-fold more) relative to the treatment groups. The cumulative survival proportion for both treatment groups was approximately 30%.

At the beginning of the experiments, a heating pad and a lamp were used to preserve body temperature at 36.5±0.5°C. After LPS administration, all groups showed a continuous increase of body temperature. After resuscitation, body temperature decreased in both treatment groups (data not shown).

Table II shows values of time, PCO_2 gap, pH_i and EIIC at the beginning of the intervention for each treatment group. Values are shown as mean and standard deviation. Throughout the intervention time, the therapy protocol guided by splanchnic tissue indicators triggered the resuscitation actions earlier than the therapy guided by

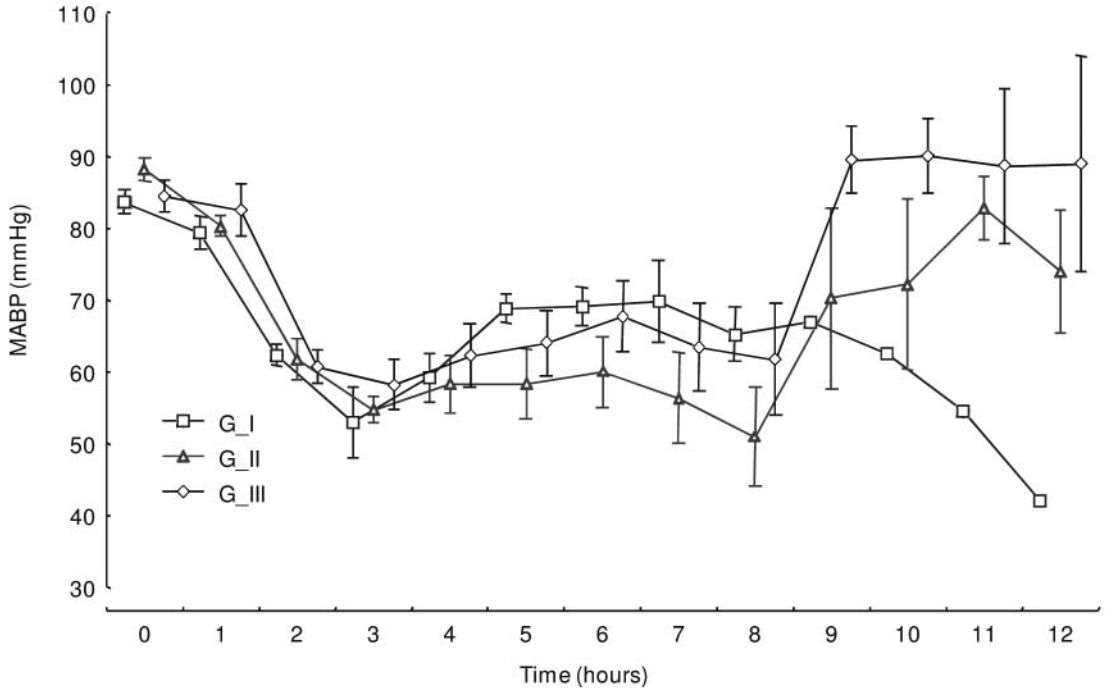

Figure 5. *Temporal course of MABP, a partial recovery of the MABP in the control group is clear after five hours in shock. There was an evident recovery of MABP in the treatment groups during the therapy time (four hours after LPS infusion). ANOVA: did not show statistical differences.*

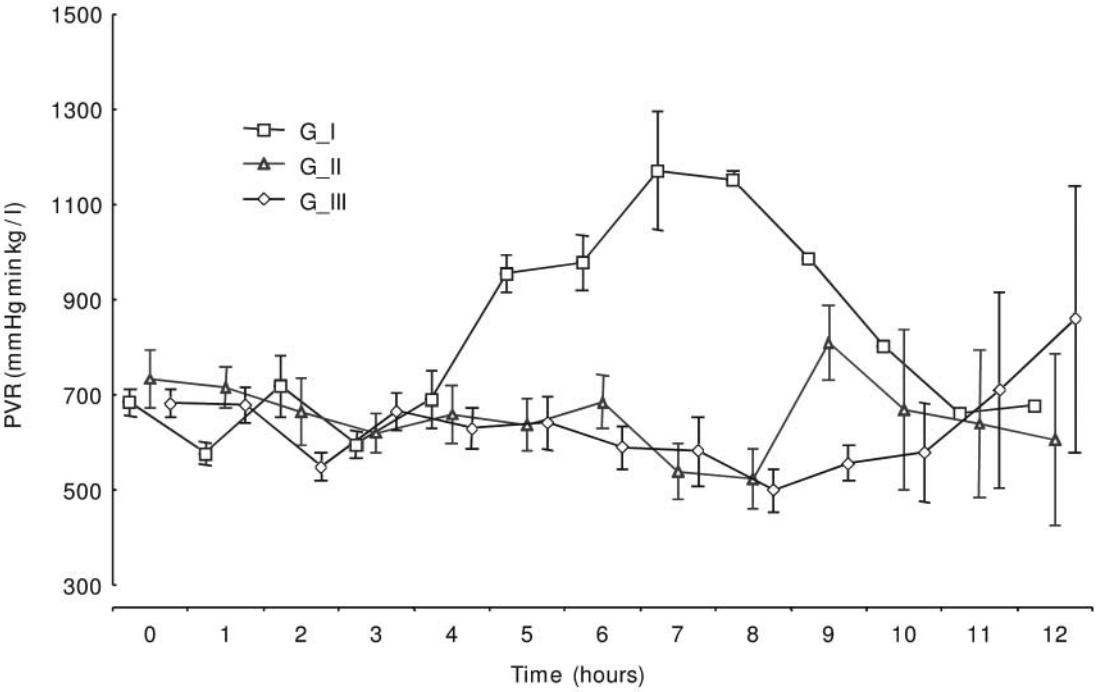

Figure 6. *Temporal course of PVR. The control group maintained a bigger PVR after 5 h. Treatment groups ran the same course than the control group for eight hours. ANOVA: $p<0.05$ with respect to control group, after 1 h beginning intervention therapies and during 7 h of resuscitation (between $t=5$ h and $t=12$ h).*

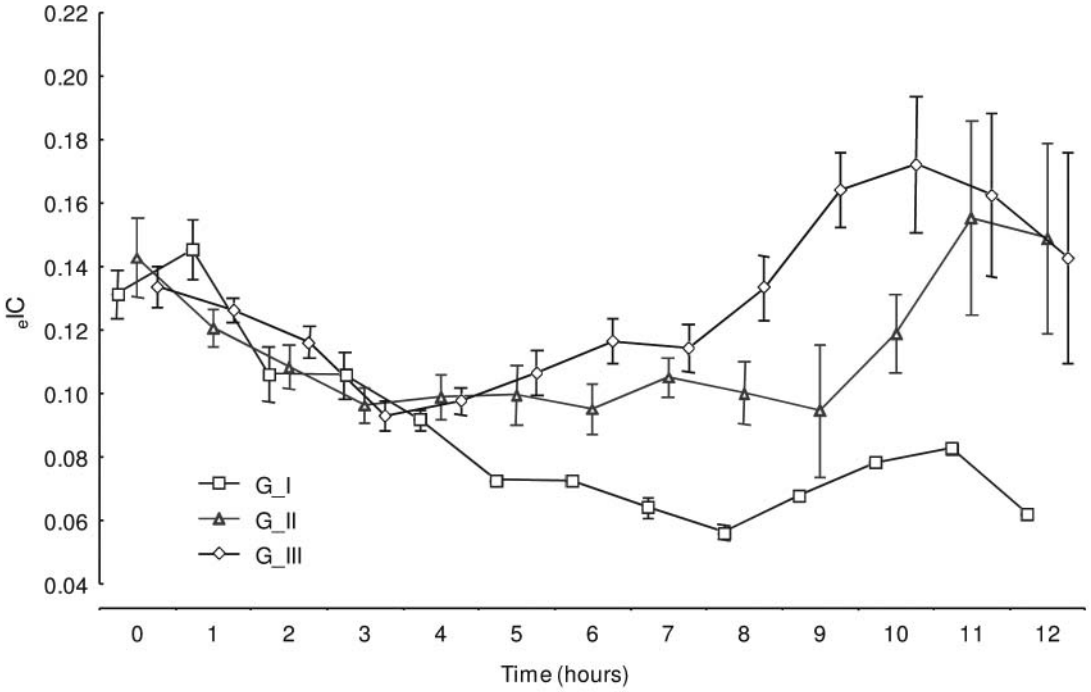

Figure 7. *Temporal course of $_e$CI. $_e$CI decreased after the infusion of LPS in all groups and in t=4 h, the value was approximately 0.1. At t=6 h, group III exhibited an improved recovery with respect to group II. The $_e$CI in the control group continued to decrease, and after 8 h showed an apparent recovery corresponding to one animal. ANOVA: p<0.05 with respect to control group, after 2 h beginning intervention therapies and during 6 h of resuscitation (between t=6 h and t=12 h).*

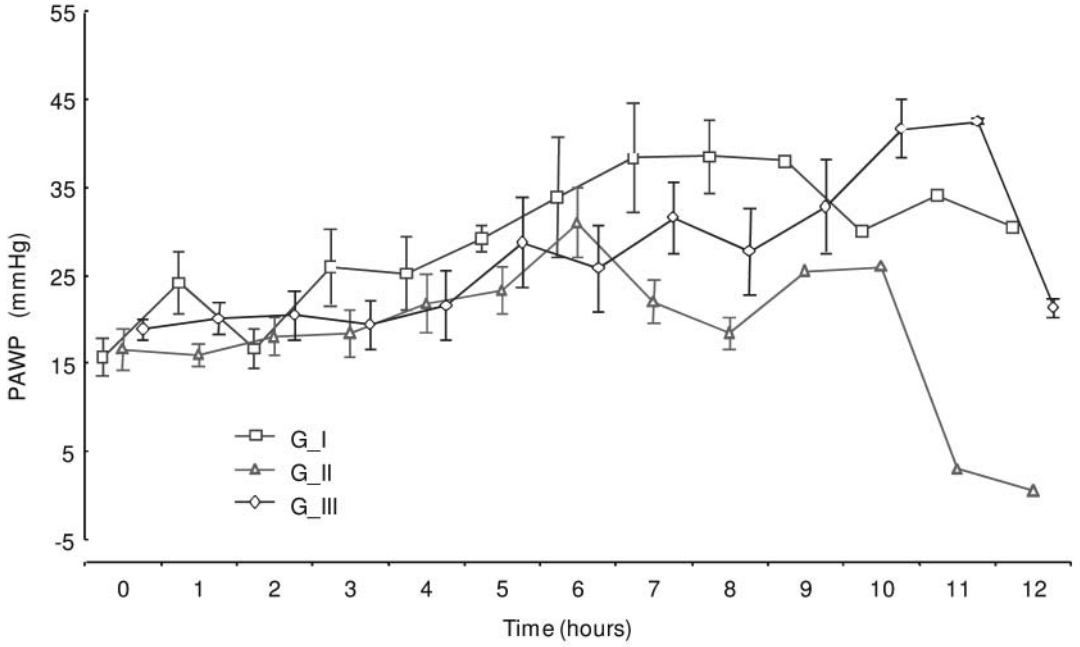

Figure 8. *The PAWP showed a continuous increase in the three experimental groups although this was less evident in the treatment groups; after 8 h, there were obvious differences between the three groups. ANOVA: did not show any statistical differences.*

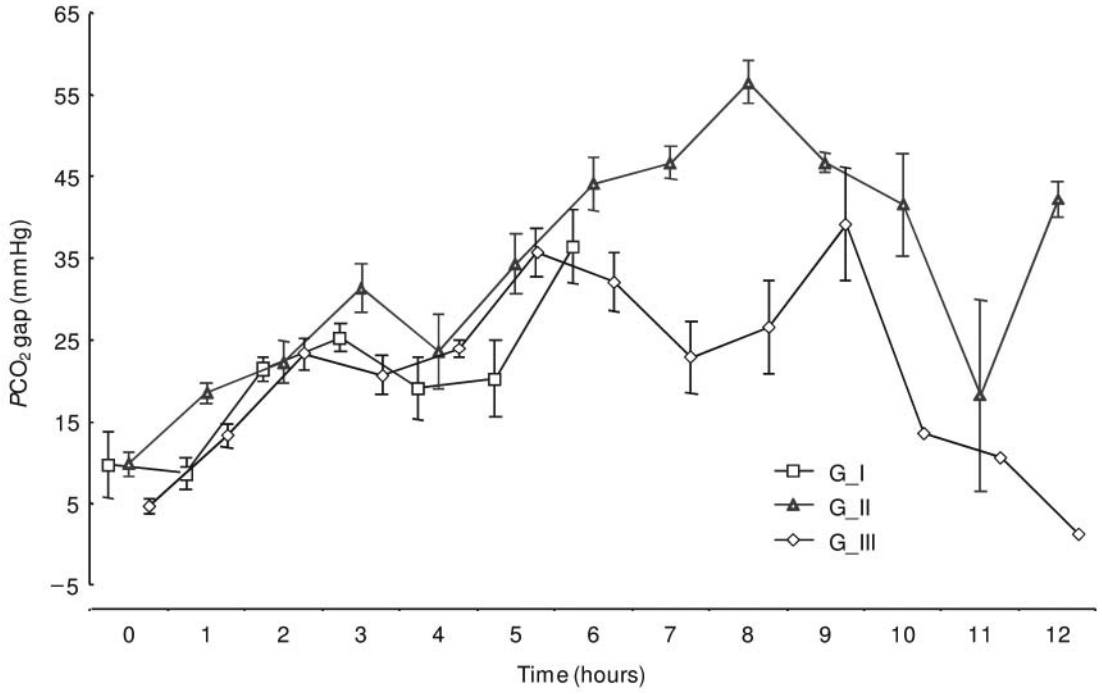

Figure 9. *Graphic representation of PCO_2 gap. After 2 h, there was already marked ischemia, and once resuscitation protocols began (after 4 h), the condition of group III improved more than that of group II. After 4 h, the PCO_2 gap data of the control group were not sufficient to identify differences among treatment groups. ANOVA: $p<0.05$ with respect to treatment groups, after 2 h beginning intervention therapies and during 2 h of resuscitation (between $t=6$ h and $t=8$ h).*

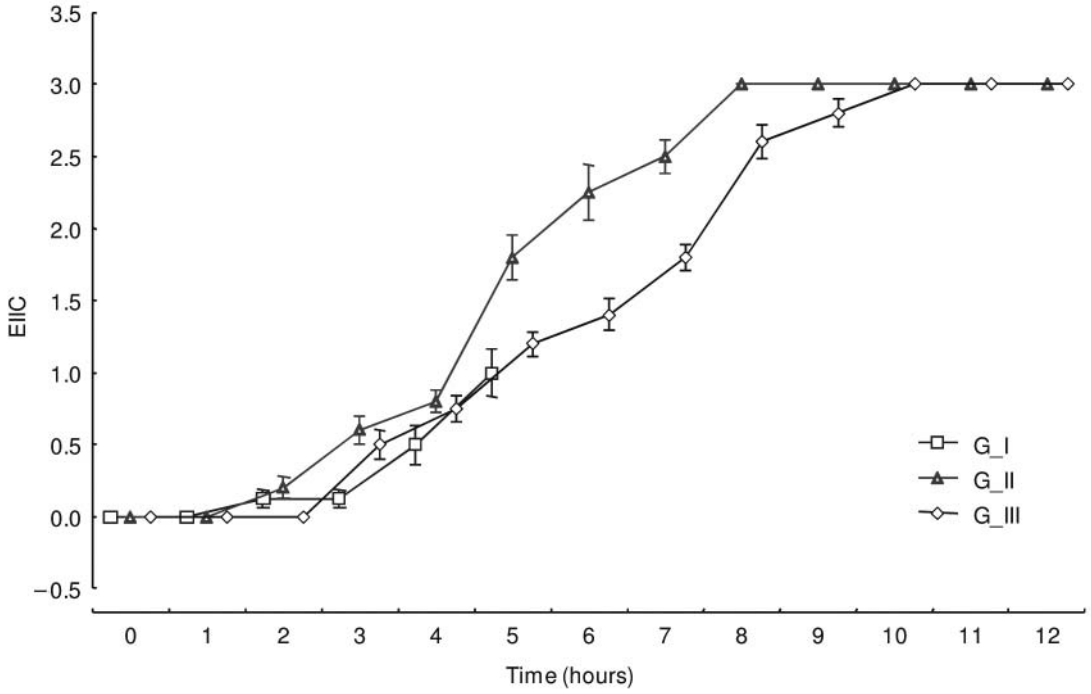

Figure 10. *Graphic representation of EIIC. Once resuscitation protocols began (after 4 h), the condition in group III improved more than that of group II. At the end of the experiment both treatment groups had EIIC values close to 3. The EIIC data of the control group after 4 h were not sufficient to identify differences among treatment groups. The EIIC ANOVA: $p<0.05$ with respect to treatment groups, at the beginning of intervention therapies and during 3 h of resuscitation (between $t=5$ h and $t=8$ h).*

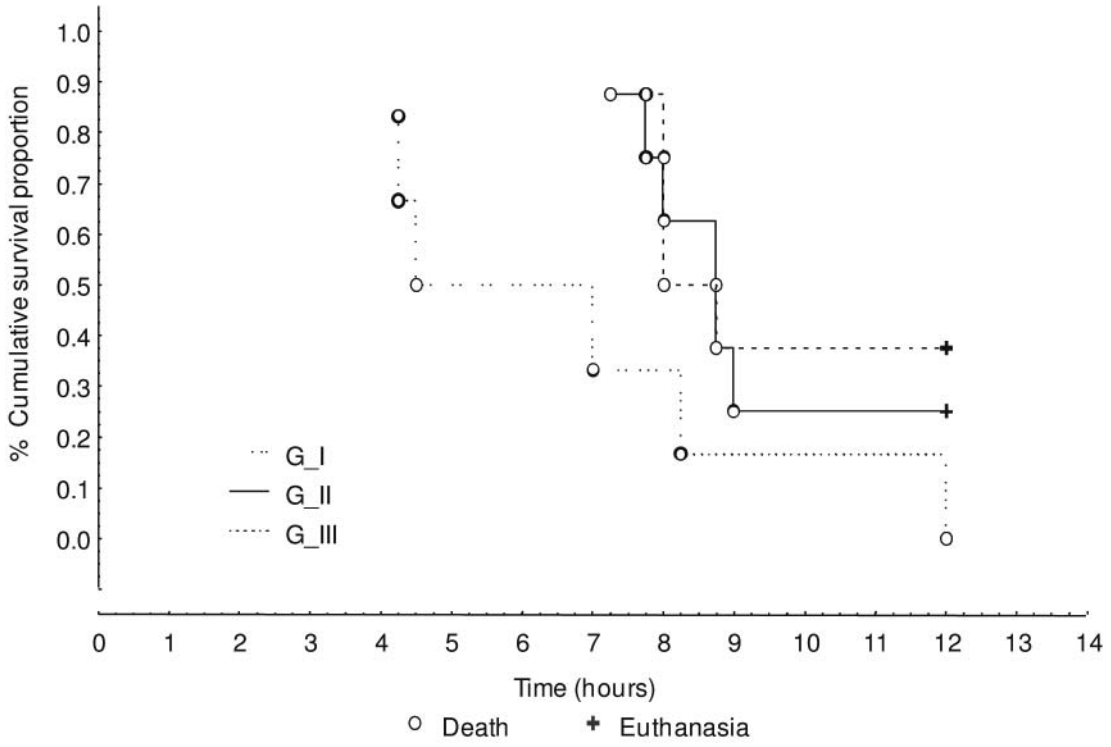

Figure 11. *Cumulative survival proportion estimated by the method of Kaplan-Meier limited product, a test of logarithm range (assuming a χ^2 distribution) provided a risk rate of the control group approximately 2.4-fold higher than treatment groups.*

haemodynamic variables. Once the therapy protocols began, the initial values of PCO_2 gap and pHi indicated a less acidotic level in group III than in group II. This fact was reflected in the EIIC values among groups II and III, which showed significant statistical differences ($p<0.05$).

At the beginning of the intervention, the recovery in haemodynamic variables and PCO_2 gap occurred faster in group III than in group II. After 4 h of intervention, these same variables exhibited a comparable improvement in recovery. Moreover, these results concurred with the 30% survival outcome that was observed in both treatment groups.

Discussion

In the three experimental groups tested, there was an evident shock condition occurring, as demonstrated by the presence of such a low MABP (60 mmHg), as well as a fall in the systemic vascular tone, from the onset of LPS administration to a time-point of t=2 h post LPS infusion. Subsequently (t=2 h), a compensation of the vascular tone ensued (reflected by the PVR increase), which denotes a correct development of the septic shock model. At the beginning of treatment time (t=4 h), there was an evident recovery of MABP in the treatment groups due to the resuscitation protocols. The control group showed a partial recovery of the MABP, but it was maintained by a high PVR. The $_eCI$ and PCO_2 gap values improved in the treatment groups, indicating that the resuscitation protocols were appropriate, at least at the beginning of the intervention.

The decrease of PCO_2 gap in group III was possibly due to impaired mucosal perfusion and with early infusion of fluids. This result is consistent with the findings of Silva E *et al.* (53). Nevertheless, a decompensation could be observed after approximately 3 hours of resuscitation with fluids and catecholamines. In this sense, during the first three hours of treatment, the PAWP had a continuous increase and its high values correlated with low values of $pH_a=7.1\pm0.1$ and $P_aO_2=80\pm10$ mmHg (data not shown), as well as with the onset of death (between 7 and 8 h). More than likely, a high PAWP with low values of $_eCI$ denotes the onset of cardiac failure, perhaps because of volume excess, or the effect of the myocardic depressor factor, or both. In addition, arterial acidosis and low P_aO_2 are associated with conditions of hypoperfusion and pulmonary edema if not attended to. The resuscitation protocols (Figures 2 and 3) did not consider a fluid limit, so possibly, pulmonary edema was induced by fluid excess,

which caused breathing impairment and hypoxia. These conditions could have promoted the high mortality observed in the treatment groups after 3 h of resuscitation.

We observed certain fluctuations in the PCO_2 gap during the final 2 h of the experimental time-course in the intervention groups. These values were consistent with a low PCO_2 gap value for one subject in group II at t=11 h. A transient improvement for just one experimental subject at that particular time seems quite unlikely because the pattern of the aforementioned values was not reflected in any of the haemodynamic variables or EIIC measurements. Hence, the results in PCO_2 gap value from this subject seem to reflect an unexpected variability of the tonometer system. In contrast, the EIIC values increased in all groups relative to basal levels, and moreover, prior to t=8 h, both treatment groups exhibited EIIC values close to 3. Since EIIC means represent a measurement of the electrical properties associated directly with the tissue status, and the PCO_2 gap represents a difference between partial gas pressures associated with an acidosis level in the tissue, the apparent discrepant pattern between the PCO_2 gap and EIIC in the intervention groups at the end of the experiments suggests that EIIC could reflect a more stable parameter related to the health status of the tissue.

The splanchnic tissue indicators allowed us to guide a resuscitation protocol by earlier measurements in the gastric tissue, which were associated with the decompensation process in shock (5) and the MOF (4). Since splanchnic tissue indicators link gastric tonometric data, this finding is consistent with previous reports suggesting that pH_i and PCO_2 gap values can be considered as an early warning of inadequate perfusion (19, 20).

In group III, the EIIC remained close to 3 even with low PCO_2 gap values for the last 3 h of each experiment, indicating that the pH_i does not necessarily reflect ischemic damage. In an earlier study (28) we concluded that the dynamic changes in impedance spectroscopy were not associated directly with either tissue perfusion or pHi, but rather, were strongly related to the duration of ischemia. Taken together, these findings suggest that impedance spectroscopy can be a useful indicator of ischemic injury as a function of the duration of ischemia. Nevertheless, it is still necessary to correlate the different levels of tissue injury obtained from the impedance spectra with the pathophysiological changes actually occurring within the gastrointestinal wall. The design of this study assumes that the impedance spectroscopy measurements reflect tissue damage. And furthermore, the results obtained have allowed us to establish that the information provided can be significantly relevant for treatment. This study was not intended to validate impedance spectroscopy and therefore has not included histological evaluation data, which at this point could have confounded experimental protocols.

In the intervention groups, the PCO_2 gap, pH_i and EIIC mean values at the time of intervention appeared to have been different; however, a *t*-test for independent samples showed statistical differences only for EIIC values. This fact has suggested that impedance spectroscopy can be a useful tool providing relevant information about the level of ischemic damage in the gastrointestinal mucosa and not just as an early warning of inadequate perfusion. However, it is important to emphasize that the experimental measurement method used is dependent on both the validation of this particular application of impedance spectroscopy as well as on the artificial neural network for spectral analysis used. As far as the authors are concerned, gastric impedance spectroscopy and neural networks have not been studied before as a complementary guide tool for resuscitation in septic shock. This study was intended to provide the first experimental data to design an optimal experimental protocol aimed at validating the impedance spectroscopy and neural networks methodology for clinical applications.

The high mortality observed after 8 h of resuscitation probably indicates that the therapeutic protocols used in this study were not the most appropriate. According to experimental results, further experiments are required to improve therapeutic protocols; for instance, as an alternative resuscitation strategy, one could consider limiting the use of catecholamines and fluids applied relative to the degree of ischemic damage observed. Moreover, adding or substituting other therapeutic modalities should also be considered. Specifically, in order to determine the optimal conditions of fluid infusion and catecholamine dosage that are required, a limited experimental study is warranted to identify how gastric impedance spectroscopy correlates with interstitial/cellular edema, membrane disintegration (*i.e.* irreversible changes) and fluid reperfusion.

Although the pigs in both treatment groups showed approximately the same cumulative survival proportion, after t=12 h the therapy guided by splanchnic tissue indicators was more effective in improving the haemodynamic conditions than the therapy guided by haemodynamic variables, albeit consisting of a small sample size (at t=12 h). An analysis of the cumulative survival proportion by a test of logarithm range did not show statistical differences among experimental groups. The risk rate of the control group was approximately 2.4-fold higher than treatment groups. In this sense, it is clear that both resuscitation protocols improved the outcome of the treatment groups and appear to have a similar experimental therapeutic value.

Conclusion

The results obtained in this study do not provide conclusive evidence that can be directly applied to clinical practice, nor was this study designed to provide such evidence. However,

the results of this preliminary study show that the information obtained, particularly by the splanchnic tissue indicators, using a minimally invasive intestinal catheter, are as sensitive as the commonly used haemodynamic variables. Therefore, this type of information in a minimally invasive non-vascular setting could serve as a relevant guide in the treatment of the critically ill during septic shock. Based on these preliminary results, further studies are required in order to develop and validate specific therapeutic guides for the critically ill. These guides would subsequently aid in the appropriate monitoring and modulation of splanchnic perfusion in critically ill patients undergoing septic shock.

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