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A comparison of the effect of convection against diffusion in hemodynamics and cytokines clearance in an experimental model of septic shock.

--Manuscript Draft--

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Corresponding Author:	Manuel E. Herrera-Gutierrez, PhD Complejo Universitario Carlos Haya Malaga, Malaga SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Complejo Universitario Carlos Haya
Corresponding Author's Secondary Institution:	
First Author:	Manuel E. Herrera-Gutierrez, PhD
First Author Secondary Information:	
Order of Authors:	Manuel E. Herrera-Gutierrez, PhD
	Gemma Seller-Pérez, PhD
	Dolores Arias-Verdú, PhD
	Maria M Granados, PhD
	Juan M Dominguez, PhD
	Rocio Navarrete, PhD
	Juan Morgaz, PhD
	Rafael Gomez-Villamandos, PhD
Order of Authors Secondary Information:	

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Basil A. Pruitt

Editor of the Journal of Trauma

Dear Dr Pruitt:

We submit the revised manuscript titled “A comparison of the effect of convection and diffusion in hemodynamics and mediators clearance in a experimental model of septic shock” for consideration for publication as Original in the Journal of Trauma.

Because a deep change of introduction and discussion was asked for we could not mark the changes in the manuscript but we have considered all the suggested changes and have sent the manuscript to a professional editing servicing for English improvement. A detailed point-to-point answers to reviewers follows.

Reviewer Comments:

Reviewer #1: This limited study looks a a form of renal replacement therapy after E coli injection in an acute model. I have the following comments:

1. This is an acute experiment that encompasses only 6 hours. The time should be measured in days.

You are absolutely correct in that this an important drawback of our study. We were limited because facilities and so it is not possible to address this problem. Anyway we believe our results are valid nevertheless because clinical studies on pulse hemofiltration show that in this window of time positive results on hemodynamics can be evidenced. Inasmuch, the original paper that we used as base to develop our model showed positive results as well. So, would the effect of diffusion be the same than convection, we should expect the same results for both groups at the end of the pulse session. We have detailed this in the discussion.

2. There were no antibiotics administered. They should have been provided

The model was based on the use of lipopolisacharides and not in whole bacteria, so we firmly believe the antibiotic could not interfere positively in

the experiment and could introduce a new uncertainty. On the other side, being this a new variation of a known model we had to be sure of the exact effect of the LPS in the control group and so we opted for the avoidance of any other treatment in our animals.
We have detailed this in the discussion.

3. There are a number of syntax errors in the manuscript which need correcting.

We have sent the manuscript to a professional editing service (this was the cause for the delay in the presentation of the new manuscript, our apologies)

4. There needs to be a better definition in the introduction on various forms of renal replacement therapy, on convection and diffusion and their major differences and how they relate to such modes as CVVH or CAVH.

We have included these concepts in the introduction

5. Heparin should have been used in the sham group since it was used in the other arms and has questionable benefit.

This has been already discussed in the question # 2, and remarked in the discussion.

6. In the discussion an increase in SVR is related to a decrease in temperature, which may only be a partial explanation as shock and bactermia cause vasodilation.

We did not make this paragraph clear… we were not discussing exactly our results but a positive effect that has been published previously. We now have reformulated these sentences because we have shortened substantially the discussion (as susgested)

Reviewer #2:

This is an LPS model which has been shown to not be relevant to most clinical sepsis. The authors need to note this limitation in the intro and discussion. The discussion is too long and should be cut in half. Figure 2 has too much data...would cut time points to 3 or 4 and present these data. A summary of the others could be presented in the text.

Yes, you are correct. Already we had mentioned this in the discussion but now we have noted this in the introduction as well.

We have shortened substantially the discussion (from 1135 to 618 words)

We have changed the graphs and the results accordingly

Corresponding author:

Manuel E Herrera Gutiérrez, PhD

ICU, Hospital Carlos Haya

Avd Carlos Haya s/n

29010, Málaga, Spain

mehguci@gmail.com

Best regards

BACKGROUND. Replacement therapies based on the use of convection have value for the removal of inflammatory mediators. Such therapies have been proposed for the management of septic shock but diffusion has not proved useful in this scenario, unless high-flow membranes are used. The exact role of diffusion in these cases remains to be clarified because continuous replacement therapies are usually delivered with low-flow membranes and mixed convection-diffusion modalities. However, studies specifically addressing this problem have not been performed. Our aim was to define the efficacy of hemofiltration (convection) and hemodialysis (diffusion) in cytokine clearance and hemodynamic improvement in an experimental model of septic shock.

METHODS: Shock was induced in 15 beagle dogs (weight 10–15 kg) by infusion of 1 mg/kg of ultrapure *E. coli* lipopolysaccharide (LPS) diluted in 20 mL saline for 10 min. Five animals were followed without interventions (controls), 5 were treated with convection (100 mL·kg^{-1}·h^{-1}) for 6 h, and 5 were treated with diffusion (100 mL·kg^{-1}·h^{-}) for 6 h.

RESULTS: All subjects in the control group died during the study, while all treated subjects survived. Mean arterial pressure, cardiac output, systolic variability volume, systemic vascular resistances, dPMax, and pulmonary compliance improved in treated subjects. However, the differences in MAP and CO were significant only in the convection group and not in the diffusion-treated group.

TNFα rose equally in all groups and decreased only in treated subjects. IL-6 rose in the 3 groups but decreased only in the convection group and remained unchanged in the control and diffusion groups.

CONCLUSIONS: Convection and diffusion improved survival and hemodynamic parameters in a septic shock model. Improvement was more pronounced with convection, a difference that may be explained by convective clearance of cytokines.

KEY WORDS: Septic shock, hemofiltration, hemodialysis, convection, diffusion.

Title:

A comparison of the effect of convection against diffusion in hemodynamics and cytokines clearance in an experimental model of septic shock.

Short title:

Is dialysis adequate to manage septic shock states?

Authors:

1 Manuel E. Herrera-Gutiérrez, PhD, mehguci@gmail.com

1 Gemma Seller-Pérez, PhD, gemmaseller@gmail.com

1 Dolores Arias-Verdú, PhD, lolaverdu@hotmail.com

2 Maria M. Granados, PhD, pv2grmam@uco.es

2 Juan M. Dominguez, PhD, pv2grmam@uco.es

2 Rocío Navarrete, PhD, pv2grmam@uco.es

2 Juán Morgaz, PhD, pv2grmam@uco.es

2 Rafael Gómez-Villamandos, PhD, pv1grmam@uco.es.

1 Cuidados Críticos y Urgencias, Hospital Universitario Carlos Haya, Málaga.

2 Departamento de Medicina y Cirugía Animal. Universidad de Córdoba.

Conflict of interest:

Author Manuel E. Herrera-Gutiérrez has received honoraria for lectures from Baxter, Fresenius, Bellco, Hospal, Astellas. For the remaining authors, no conflicts are declared.

This research was funded by a non-restricted grant from Hospal®.

Presentation in meetings:

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Abstract

BACKGROUND. Replacement therapies based on the use of convection have value for the removal of inflammatory mediators. Such therapies have been proposed for the management of septic shock but diffusion has not proved useful in this scenario, unless high-flow membranes are used. The exact role of diffusion in these cases remains to be clarified because continuous replacement therapies are usually delivered with low-flow membranes and mixed convection-diffusion modalities. However, studies specifically addressing this problem have not been performed. Our aim was to define the efficacy of hemofiltration (convection) and hemodialysis (diffusion) in cytokine clearance and hemodynamic improvement in an experimental model of septic shock.

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LEVEL OF EVIDENCE: Level III study.

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Background

Severe sepsis during the evolution of uncontrolled infection is the leading cause of multiple organ dysfunction syndrome (MODS) in the intensive care unit (ICU) and a main cause of mortality (1,2). An adequate and early approach to its management can alter its course and improve the prognosis (3). Molecules expressed in the bacterial membrane activate host defense mechanisms by the production of different pro- and anti-inflammatory molecules (e.g., the cytokines TNFα, IL-B1, or IL-6) that initiate an inflammatory cascade (4). In this context, the use of a depurative technique to eliminate pro-inflammatory molecules and halt the development of septic shock and multiple organ failure (MODS) is attractive. Depuration can be achieved by applying convection (molecules are displaced following the movement of water when a pressure gradient is generated in a semipermeable membrane), diffusion (molecules pass through the membrane via a concentration gradient), or adsorption (molecules are trapped in the membrane). The first case is known as hemofiltration (HF) and is able to clear molecules of low and medium molecular weight; the second case is known as dialysis (HD) and can clear only molecules of low molecular weight.

Aggressive convective therapies such as high volume hemofiltration (HVHF) or adsorption (5-8) have been used in sepsis. It has always been assumed that cytokines are out of the reach of dialysis because their molecular weight (this axiom has recently been challenged with the development of high cut-off membranes) (9-12). However, some groups have demonstrated that combining diffusion with convection improves prognosis in septic patients (13). However, convection is useful for clearing cytokines only when high doses are applied (HVHF) (14). Due mainly to technical reasons, semi-synthetic membranes are not suited for diffusion at high doses (15). Therefore, while much experience has been gained in experimental and clinical studies on the use of high-dose convection for clearing cytokines, analysis of the exact role of diffusion has not been published to our knowledge.

Evidence for cytokines clearance has been obtained mainly in experimental septic shock. However, animal models behave differently to humans, and this difference is more relevant when using lipopolysaccharide (LPS) injections. Nonetheless, animal models that were previously used to define the role of HVHF in the septic shock could be translated into clinical practice. Therefore, we opted for a model based on LPS and designed a protocol to test the (until now unexplored) role of diffusion using a conventional semi-synthetic membrane on cytokine clearance and hemodynamic parameters, by comparison to an equal high-dose convective therapy.

Methods

Subjects and groups of study

We included 15 beagle dogs distributed into 3 groups: 5 subjects that did not receive treatment (controls); 5 treated with a technique based on convection (high volume hemofiltration – HVHF); and 5 treated with a technique based on diffusion in an equal dose to the HVHF group (continuous hemodialysis – CHD). The mean weight of the subjects was 12.56 ± 0.73 kg, which corresponded to 12 ± 1.22 kg in the control group, 13.76 ± 1.3 kg in the HVHF group, and 11.5 ± 1.04 kg in the CHD group.

Anesthetic protocol and monitoring

We applied the clinical protocol used in this center: gas anesthesia, atracurium relaxation, and ventilation aimed at normal end-tidal CO_2. A Picco ® arterial catheter was inserted into the femoral artery, and a pulmonary catheter was inserted into an internal jugular vein together with a Datex Ohmeda ® (GE Healthcare) gastric mucosal

CO_2 probe. For subjects in the HVHF and CHD groups, a 12-F double lumen catheter was inserted into the other internal jugular vein.

Cytokine assays

Blood samples were placed into sterile anti-coagulant free tubes and centrifuged at 4,000 rpm for 15 min at room temperature (20-22°C). The serum was collected aseptically and stored at -80°C until use. All assays were performed in duplicate. TNFα and IL-6 levels in serum were evaluated using commercial quantitative solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) kits (Canine TNFα/TNFSF1A Immunoassay and Canine IL-6 Immunoassay; R&D Systems Europe Ltd., United Kingdom) according to the manufacturer's instructions. Standard curves to calculate cytokine concentrations (pg/ml) were generated using recombinant canine IL-6 and TNFα provided in the kits. The final absorbance of the samples was measured with a microliter plate reader at 450 nm with a Ceres UV 900® spectrophotometer (Bio-Tek Instruments), and a wavelength correction set at 540 nm or 570 nm.

Septic shock provocation

A model developed by our group and previously published in detail (16) has been applied. With the subjects anesthetized, monitored and in a stable condition, we infused 1 mg/kg of body weight of ultrapure *Escherichia coli* lipopolysaccharide (strain 0111:B4, InvivoGen ®) diluted in 20 mL of saline solution for 10 min.

Extracorporeal therapy protocol

Fifteen minutes after the end of the LPS infusion, extracorporeal therapy was initiated in the HVHF and CHD groups according to the same protocol. Connection was performed in both lumens simultaneously and a single dose of 1 mL/kg of hydroxietilstarch was infused to compensate for blood loss in the circuit. Apart from extracorporeal therapy, this was the only intervention that was performed during the whole study. A 0.9 m^2 AN69 membrane was used that was attached to a Prisma® (Hospal) with a blood flow of 130 mL/min, zero negative balance, bicarbonate buffered fluids and anticoagulant based on non-fractionated heparin 15 $U \cdot kg^{-1} \cdot h^{-1}$. To control the temperature, the lines were inserted in a warm bath and a thermic blanket was used. The subjects in the HVHF group received a dose of 100 $mL \cdot kg^{-1} \cdot h^{-1}$ of convective treatment and zero dialysis. The subjects in the CHD group received a dose of 100 $mL \cdot kg^{-1} \cdot h^{-1}$ of diffusive treatment and zero convection.

Ethical issues

This study carried was out in an experimental operating room of the Veterinary Hospital of the University of Córdoba, Spain. Survivors at the end of the follow-up were euthanized following the standard measures approved for this Veterinary Hospital. The Ethics and Clinical Research Committee at the Veterinary Hospital of the University of Córdoba approved the protocol.

Statistical analysis

Data are shown as mean ± mean standard error or proportions and rounded to one decimal precision. Comparisons were performed with the Mann-Whitney or Kruskal-Wallis test, with a level of significance for all tests of 0.05.

Results

All subjects in the control group died during the study: 1 at 150 min, 1 at 250 min, 1 at 315 min, and the other 2 at the end of follow-up (360 min). The mean survival time was 288 ± 40 min. All subjects in the HVHF and CHD groups survived this period.

All subjects were shocked at the end of the LPS infusion (10 min), with a mean fall in mean arterial pressure (MAP) of 43 mm Hg, a mean fall in cardiac output (CO) of 0.5 L/min, and a mean rise in systolic volume variability (SVV) of 13%. An early fall in pulmonary compliance was also detected (Table 1).

Fifteen minutes after the end of the LPS infusion, hemofiltration (convection), and dialysis (diffusion) were initiated in the HVHF and CHD groups at the same fluid rate (100 $mL \cdot kg^{-1} \cdot h^{-1}$), and measures were taken hourly in the 3 groups under study.

Of the variables analyzed, MAP, CO, SVV, systemic vascular resistances (SVR), dPMax, and pulmonary compliance were significantly different between treated and control groups. Gastric mucosal CO_2 was higher and base deficit and PaO_2/FiO_2 lower in the control group, but the differences were not statistically significant. Temperature, mean pulmonary arterial pressures (MPAP), and pulmonary vascular resistances (PVR) were similar in the 3 groups (Table 2).

When comparing the behavior of the HVHF and CHD groups, the response in SVV and dPMax was similar for both groups (significantly better than in the control group). However, when analyzing MAP and CO, the differences to the control group reached statistical significance in the HVHF group but not in the CHD group (Figure 1).

IL-6 increased at 120 min in a similar fashion in all subjects and then decreased at the end of follow-up only in the HVHF group, but without reaching statistical significance. TNFα showed a similar trend, with a peak at 120 min that was equal in all subjects, and a decrease at the end of follow-up in the CHD and HVHF groups (p 0.09 for HVHF against controls) (Figure 2).

Discussion

Infection activates the production of cytokines, which trigger a response that leads to septic shock and MODS (17). Hemofiltration or adsorption (5,7,14, 18-23) have been employed to ameliorate this response, and some beneficial effects have also been reported when using convection and diffusion together (hemodiafiltration) (13,24-26). However, to our knowledge, no previous studies have been performed that analyzed the exact role of dialysis for clearing cytokines. Factors other than cytokine clearance have been considered a possible explanation for this positive effect (e.g., adsorption in the membrane, slow fluid changes, hypothermia or pH normalization) (27-30). It is therefore not surprising that we achieved a hemodynamic improvement in the dialysis group. However, the subjects treated with HVHF also gained an additional benefit in hemodynamics, for which cytokine clearance is the most likely explanation (31-33). The only recent study comparing diffusion and convection (but which did not investigate hemodynamic improvement or cytokine clearance), published by Ricci et al (34) in 2006, concluded that medium-size molecules are not cleared efficiently by diffusion, which is in agreement with our results.

We acknowledge certain methodological problems with our protocol, including the short follow-up time of the experiment. A longer study may have provided more significant results; however, clinical studies on high-volume pulse hemofiltration have been performed in 4–6 h sessions and showed a positive effect during this time frame. The study on which our animal model was based (20) also showed a positive response during this period. Therefore, if the effects of diffusion were the same as convection, we may expect the same results in both groups at the end of the session.

The use of a sham group may have determined the effect of adsorption or other factors (temperature, pH, etc.) on our results, but would not have affected the comparison because both intervention groups were subjected to the same protocol and only the principle of therapy differed.

A hypothetical role of heparin on survival after experimental shock induced by LPS has been reported (35) and this effect cannot be ruled out in our protocol. However, because our study was based on a variation of a previous model, we needed to define exactly the effect of LPS and avoided any therapeutic intervention in the control group (and subsequently in the treatment groups). Nonetheless, this should not interfere with the comparison between treatments but only with the control group.

The use of a very high rate of dialysate with a semisynthetic membrane can be questioned, but in our experience the dose of dialysis that we can achieve efficiently with our AN69 membranes is as high as 50 mL/min (36). Therefore, we included animals of low weight to maintain the dialysis below this limit while providing the target dose of 100 $mL \cdot kg^{-1} \cdot h^{-1}$.

Finally, we accept that any septic shock model will always be different to the process in humans (37), with those based on living bacteria being closer to the clinical setting (19,38,39). Considering that previous studies on the effects of hemofiltration in LPS induced septic shock (20) could be translated successfully into clinical practice (18), we opted for our chosen model (16) because of its predictable time-effect relationship. However, we acknowledge that further studies are needed to confirm its validity in the clinical setting.

When approaching a patient in septic shock who requires extracorporeal therapy, preference should be given to the use of convection (hemofiltration). Further, if the therapy is used primarily to control the shock state, diffusion may not provide an additional benefit over convection, unless a positive effect can be demonstrated in future clinical studies with the use of high cut-off membranes.

Author Contributions.

Dr. Herrera-Gutiérrez and Dr. Seller-Pérez generated the hypothesis, designed the study protocol, performed the statistical analysis and prepared the manuscript. Dr Granados, Dr. Dominguez, Dr. Navarrete, Dr. Morgaz and Dr. Gómez-Villamandos, designed the animal management protocols and performed the anesthetic procedures. All the authors collected data, reviewed the manuscript and acknowledged the contents.

Acknowledgments.

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Figure 1.- Changes in selected haemodynamic variables between groups since shock provocation until end of follow-up (six hours).
Bars represent mean standard error

Figure 2.- Changes in IL-6 and TNFα serum levels between groups since shock provocation until end of follow-up (six hours).
Bars represent mean standard error

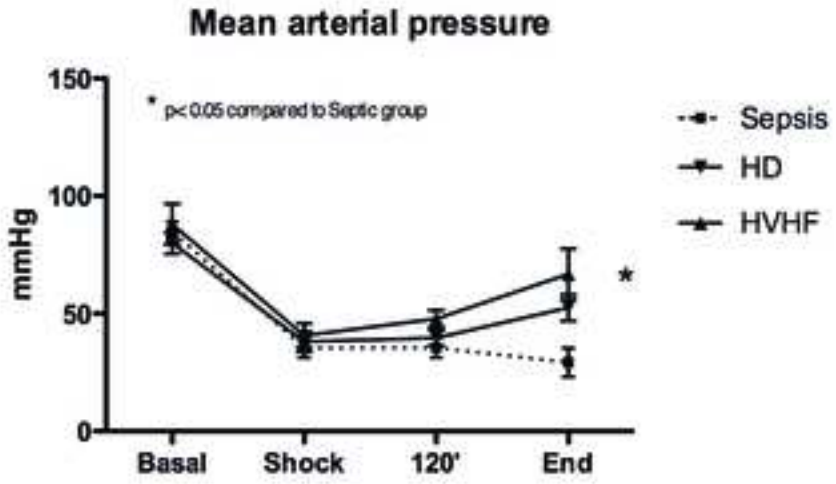
Table 1.- Provocation of shock: changes during the 10 minutes infusion of 1 mgr/Kg Escherichia Coli lipopolysaccharide. [a]Significant changes between base level and end of infusion. Data as mean ± mean standard error

	Basal	10’	p[a]
Mean arterial pressure *mmHg*	81±4	38.1±2.6	*<0.001*
Cardiac output *L/min*	1.4±0.1	0.9±0.08	*0.06*
Systemic vascular resistances *Dyne.sec/cm*^{5}	5704±497	2910±254	*<0.001*
Systolic volume variability *%*	12±1	25±1.9	*<0.001*
dPmax	666±52	326±37	*<0.001*
Central venous pressure *mmHg*	5.8±0.9	5.5±0.6	*ns*
Mean pulmonary pressure *mmHg*	12.4±0.9	12.1±0.9	*ns*
Capillary pulmonary pressure *mmHg*	7.3±1.1	5.8±0.7	*ns*
Pulmonary vascular resistances *Dyne.sec/cm*^{5}	426±79	562±90	*ns*
Gastric mucosal CO_2 *torr (kPa)*	48±4 (6.5±0.5)	54±2 (7.2±0.3)	*ns*
Temperature *ºC*	35.9±0.5	35.7±0.5	*ns*
Compliance	16.0±0.6	12.8±0.8	*<0.005*

Table 2.- Evolutive changes of selected variables during follow-up.

HVHF: hemofiltration group; CHD: haemodialysis group; control: no treated group. Data as mean ± mean standard error. ^{a}p < 0,05 for the difference at end of follow-up between control and treatment groups.

		shock	120’	last measure
Temperature *ºC*	HVHF	35,4±2,1	35,9±2,1	35,6±3,4
	CHD	35,6±1.0	33,5±0.8	33,1±1.0
	Control	35,8±1,7	34,4±0,6	34,8±0,9
^{a}Systemic vascular resistances *Dyne.sec/cm^{5}*	HVHF	2593±430	2084±310	3614±735
	CHD	2822±514	2978±1085	3721±979
	Control	3296±428	2889±667	2596±516
Mean pulmonar pressure *mmHg*	HVHF	9,4±1,3	10,9±1,7	10,6±1,5
	CHD	13,9±2.3	9,5±1.2	10,2±0.2
	Control	13,5±1,1	9,5±0,9	9,2±1,1
Pulmonary vascular resistances *Dyne.sec/cm^{5}*	HVHF	280±39	256±60	274±77
	CHD	593±271	267±105	363±18
	Control	310±116	298±59	435±70
Gastric mucosal CO2 *torr (kPa)*	HVHF	55±5 (7,3±0,6)	65±2 (8,7±0,2)	72±11 (9,6±1,5)
	CHD	59±5 (7,9±0.6)	65±4 (8,7±0.5)	68±3 (9,1±0.4)
	Control	50±5 (6,6±0,6)	103±17 (13,7±2,2)	109±10 (14,5±1,3)
^{a}Compliance *mL/cmH$_2$O*	HVHF	14,4±1,2	14,0±0,5	13,8±0,6
	CHD	12,8±2.1	13,3±1.7	13,3±1.3
	Control	11, 4±0,6	12,6±0,2	11,2±0,5
Base deficit *meq*	HVHF	-9, 0±1,0	-13,0±1,4	-10,5±3,7
	CHD	-8,6±0.5	-8,±1.53	-8,8±2.9
	Control	-14,0±1,0	-16,0±1,1	-18,9±3,2
PaO$_2$/FiO$_2$	HVHF	416±33	559±24	614±88
	CHD	376±109	357±103	490±246
	Control	487±75	464±81	461±94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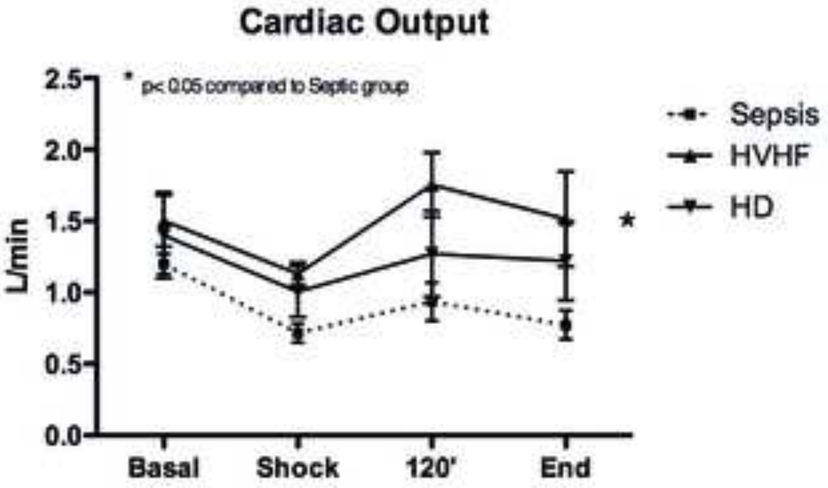
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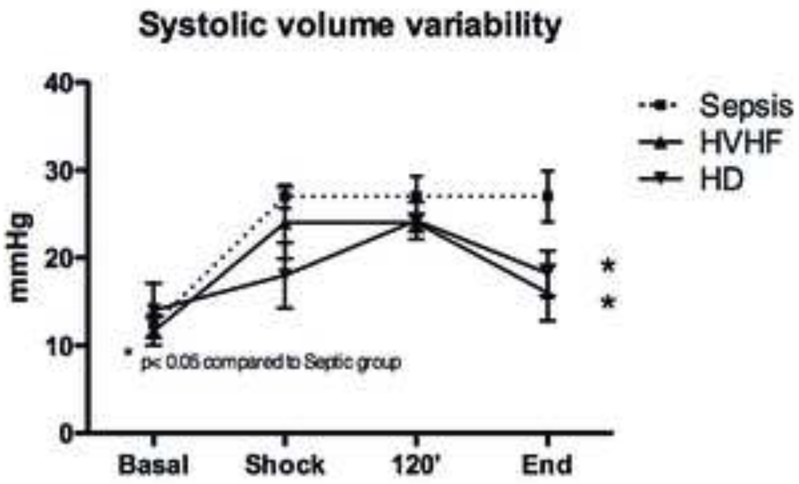
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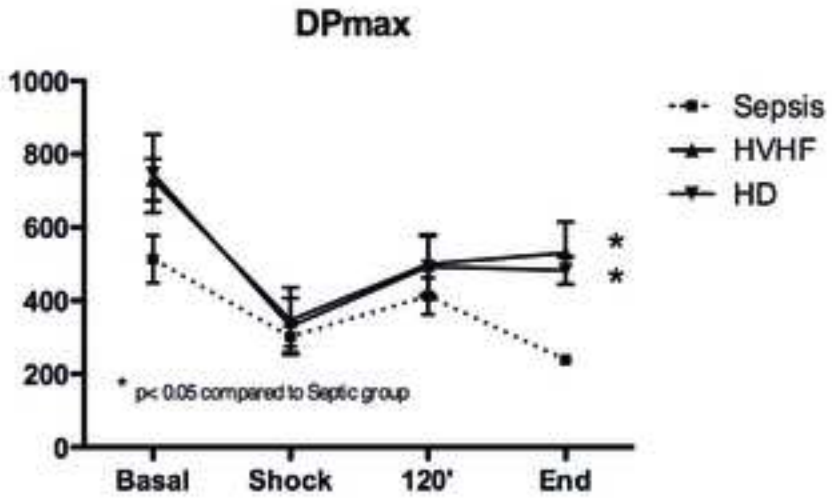
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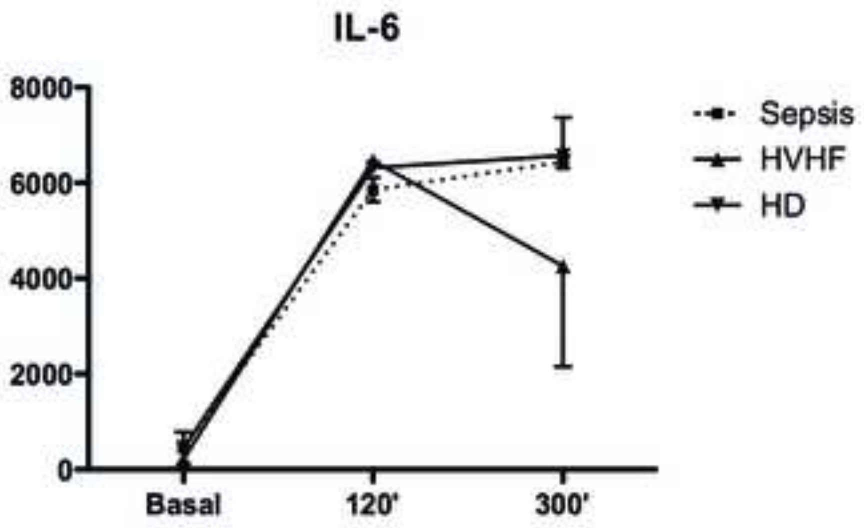
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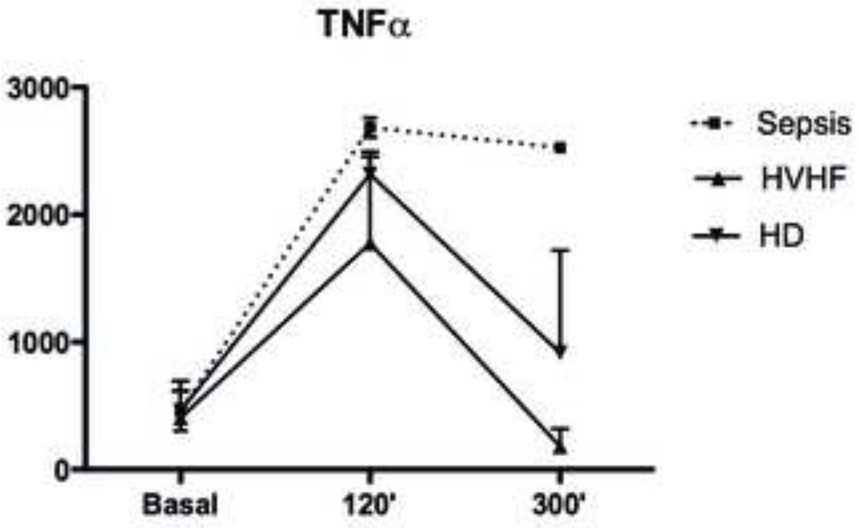
Sepsis
HVHF
HD


TNFα
3000
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Basal
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Sepsis
HVHF
HD