Supplemental Material

Data S1.

Supplemental Methods

Flow cytometry

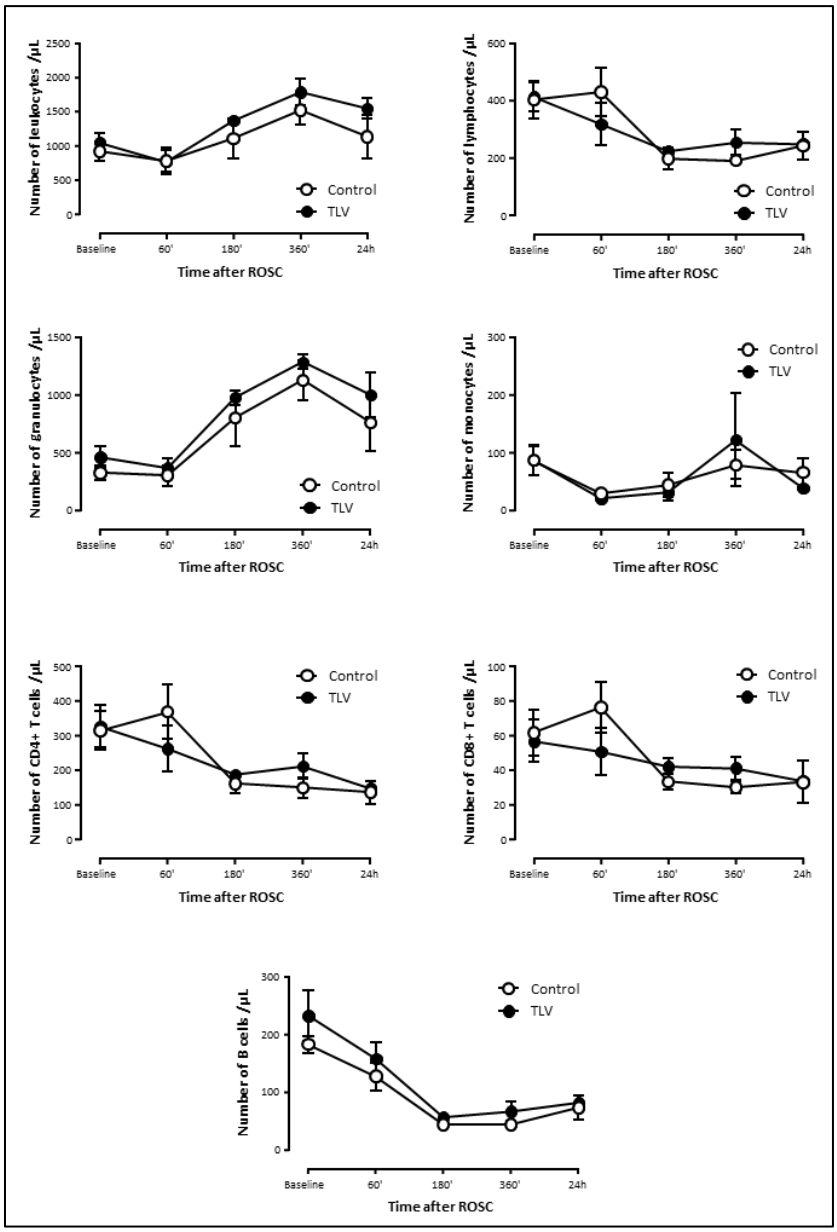
In the Control and TLV groups, 100 µL of peripheral blood samples were collected before the onset of cardiac arrest and at different time points throughout the procedure (i.e., 1 h, 3 h, 6 h and 24 h post-ROSC). Erythrocytes were lysed for 15 min, and the debris were washed away, in accordance to the manufacturer recommendations (RBC Lysing Buffer, Tebubio, Le Perray-en-Yvelines, France). Cells were then fixed in 0.5% paraformaldehyde/PBS before staining the next day. Then, cells were incubated with Fcblock, and stained using the following monoclonal antibodies: (APC)-labelled anti-T-cells marker (KEN5, 5 µL, Santa Cruz Biotechnology, Dallas, TX, USA), phycoerythrin (PE)-labelled anti-CD4 (0.75 µL, Lifespan Biosciences, Seattle, USA), fluorescein isothiocyanate (FITC)-labelled anti-CD8 (5 µL, Lifespan Biosciences, Seattle, USA), APC-labelled anti-CD11b (clone M1/70, 1 µL, EXBIO praha, Vestec, Czechoslovakia), FITC-labelled anti-CD14 : 5 µL (Clone TÜ ,5 µL, Lifespan Biosciences, Seattle, USA), APC-labelled anti-CD79a (clone HM57, 5 µL, EXBIO praha, Vestec, Czechoslovakia). To perform surface antigen staining, cell suspensions were incubated on ice with appropriate mix of antibodies for 30 min. Then, cells were washed three times with a phosphate buffered saline solution containing bovine serum albumin (0.5%) and finally resuspended in running buffer for flow cytometry. Intracellular staining with CD79-a, for B-lymphocytes identification, was performed with Thermofisher Fix & Perm cell permeabilization kit ®, according to the manufacturer's instructions. Four-color flow cytometry was conducted with the BD Biosciences AccuriC6 device.

Table S1: Blood gases and biochemical parameters throughout protocol in the different groups. Data are expressed as mean ± SEM.

*Statistical comparisons were only made for group effect but not among time-points. *, p<0.05 vs Sham; †, p<0.05 vs Control.*

Parameters and groups	Baseline	After ROSC		
		30 min	360 min	24 h
Lactate blood level (mmol/L)				
Sham	4.7 ± 1.4	3.3 ± 1.7	3.7 ± 1.7	5.0 ± 4.7
Control	5.7 ± 1.4	15.7 ± 1.4*	9.1 ± 1.5*	5.6 ±2.2
TLV	4.3 ± 0.7	16.9 ± 1.0*	7.4 ± 0.9	2.9 ± 1.2
Bicarbonate blood level (mmol/L)				
Sham	28.4 ± 3.3	26.2 ± 1.6	27.0 ± 1.6	21.2 ± 1.9
Control	28.6 ± 1.5	16.8 ± 2.4*	20.5 ± 2.9	21.2 ±2.0
TLV	27.4 ± 2.1	21.6 ± 1.4	20.8 ± 1.8	26.1 ± 3.0
Creatinine blood level (µmol/L)				
Sham	67 ± 11	59 ± 27	81 ± 14	85 ± 10
Control	62 ± 12	158 ± 53	107 ± 39	136 ± 45
TLV	53 ± 8	121 ± 39	82 ± 8	88 ± 23
Glucose blood level (mg/dL)				
Sham	1.8 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
Control	1.7 ± 0.1	3.9 ± 0.1*	3.4 ± 0.8*	1.7 ± 0.2
TLV	2.0 ± 0.1	4.1 ± 0.3*	3.1 ± 0.4*	1.3 ± 0.1

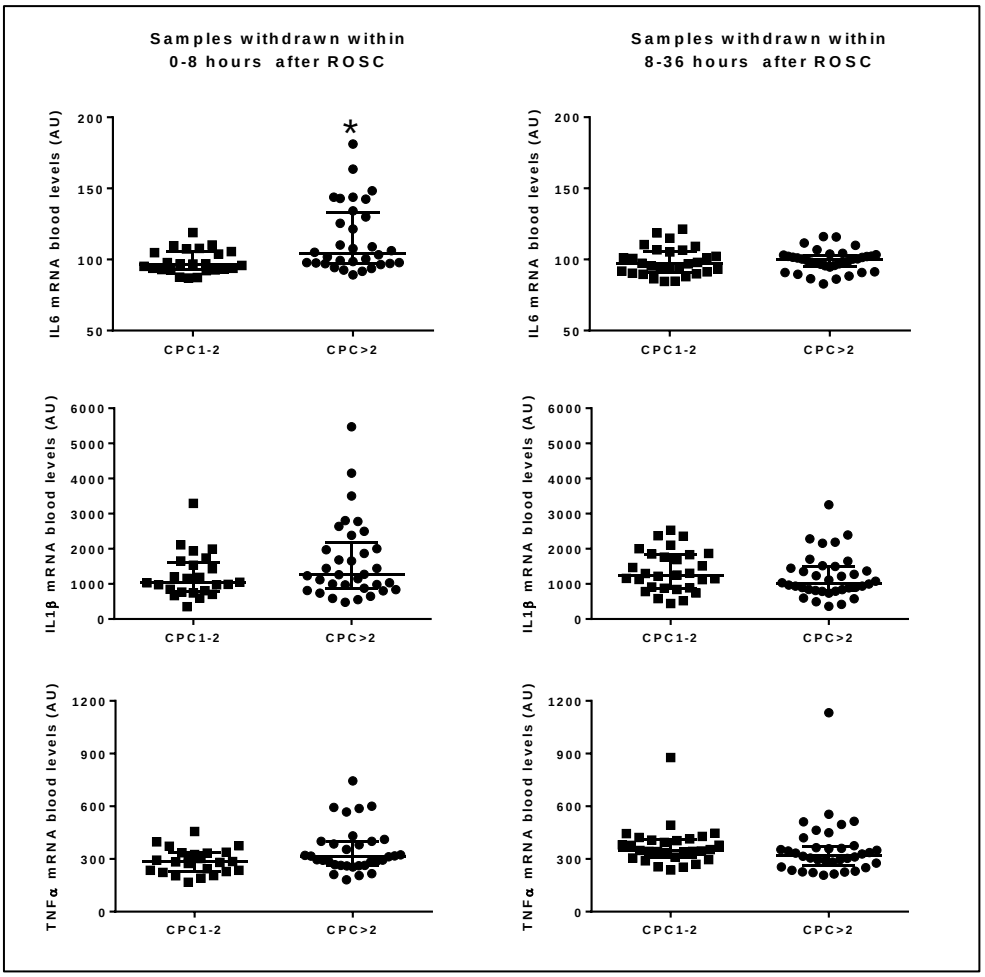
Figure S1.Leukocytes counts in the peripheral circulation throughout the experimental protocol in the different groups.



Granulocytes were considered as $CD11^{+}CD14^{-}$ cells, monocytes as $CD11^{+}CD14^{+}$ cells and B cells as $CD79a^{+}$ cells. T cells were identified thanks to a specific marker and further classified as $CD4^{+}$ or $CD8^{+}$.

Data are expressed as mean±sem. Statistical comparisons were only made between groups but not among different time points; TLV, total liquid ventilation, ROSC, resumption of spontaneous circulation.

Figure S2. Blood transcription level of interleukin (IL)-6, IL-1β and Tumor Necrosis Factor (TNF)-α in patients after out-of-hospital cardiac arrest.



Transcription levels were analyzed in samples withdrawn within 0-8 h vs 8-36 h after cardiac arrest (n=58 and 73, respectively). They were compared in patients with favorable vs poor neurological outcome, *i.e.*, cerebral performance category (CPC)1-2 (n=25 and 33 for patients sampled <8 h and between 8-30 hours, respectively) *vs* CPC>2 (n=28 and 35 patients sampled <8 h and between 8-30 hours, respectively). Open circles and bold lines represent individual and mean values, respectively.

*Data are expressed as individual (squares and circles), medians and interquartile values. *, p<0.05 vs corresponding CPC 1-2.*