RESUSCITATION 138 (2019) 222–232

Available online at www.sciencedirect.com

Resuscitation

journal homepage: www.elsevier.com/locate/resuscitation

Clinical paper

Early blood transcriptomic signature predicts patients' outcome after out-of-hospital cardiac arrest

Renaud Tissier[a,b,*,1], Hakim Hocini[a,c,1], Nicolas Tchitchek[c,d], Nicolas Deye[e], Stéphane Legriel[f], Nicolas Pichon[g], Cédric Daubin[h], Olivier Hermine[i], Pierre Carli[j], Benoît Vivien[j], Jean-Marc Tréluyer[k], Cécile Lefebvre[a,c], Pascaline Tisserand[a,c], Jean-Luc Dubois-Randé[a,b], Alain Berdeaux[a,b], Bijan Ghaleb[a,b,l], Jean-Daniel Lelièvre[a,c], Yves Levy[a,c,**,2], Alain Cariou[m,2]

[a] Inserm, U955, F94000, Créteil, France
[b] Université Paris Est, UMR_S955, UPEC, Ecole Nationale Vétérinaire d'Alfort, F-94000, Créteil, France
[c] Vaccine Research Institute, Université Paris Est-Créteil, F-94000, Créteil, France
[d] CEA — Université Paris Sud 11 — INSERM U1184, Immunology of Viral Infections and Autoimmune Diseases, IDMIT Infrastructure, F-92265 Fontenay-aux-Roses, France
[e] Medical ICU, Inserm U942, Lariboisiere Hospital, APHP, F-75010, Paris, France
[f] Intensive Care Unit, Versailles Hospital, Le Chesnay, F-78150, France
[g] Intensive Care Unit, University Hospital Dupuytren, Limoges, F-87042, France
[h] CHU de Caen, Department of Medical Intensive Care, Caen, F-14000, France
[i] Department of Hematology and INSERM U1163 CNRS ERL 8654, Imagine Institute and Necker Hospital, Paris, F-75015, France
[j] SAMU de Paris, Service d'Anesthésie-Réanimation, Hôpital Universitaire Necker— Enfants Malades, Université Paris Descartes, F-75015, Paris, France
[k] Clinical Research Unit, Paris Centre and Paris Descartes University, Paris, France
[l] APHP, Hôpitaux Universitaires Henri Mondor, Plateforme de Ressources Biologiques, F-94000, Créteil, France
[m] Service de Réanimation Médicale, Hôpitaux Universitaires Paris Centre, Hôpital Cochin, Paris, France

Abstract

Background: Early prognostication is a major challenge after out-of-hospital cardiac arrest (OHCA).

Aims: We hypothesized that a genome-wide analysis of blood gene expression could offer new prognostic tools and lines of research.

Methods: Sixty-nine patients were enrolled from an ancillary study of the clinical trial NCT00999583 that tested the effect of erythropoietin (EPO) after OHCA. Blood samples were collected in comatose survivors of OHCA at hospital admission and 1 and 3 days after

* Corresponding author at: Inserm U955, Institut Mondor de Recherche Biomédicale, Ecole Nationale Vétérinaire d'Alfort, 7 avenue du Général de Gaulle, 94700, Maisons-Alfort, France.
** Corresponding author at: INSERM U955, Institut Mondor de Recherche Biomédicale, Vaccine Research Institute, CHU Henri Mondor, 51 avenue Mal de Lattre de Tassigny, 94010, Créteil, France.
E-mail addresses: renaud.tissier@inserm.fr (R. Tissier), yves.levy@inserm.fr (Y. Levy).
[1] Both authors contributed equally to this work.
[2] Both authors contributed equally to this work.
https://doi.org/10.1016/j.resuscitation.2019.03.006
Received 19 December 2018; Received in revised form 27 February 2019; Accepted 10 March 2019

resuscitation. Gene expression profiles were analyzed (Illumina HumanHT-12 V4 BeadChip; >34,000 genes). Patients were classified into two categories representing neurological favorable outcome (cerebral performance category [CPC] = 1-2) *vs* unfavorable outcome (CPC > 2) at Day 60 after OHCA. Differential and functional enrichment analyses were performed to compare transcriptomic profiles between these two categories.

Results: Among the 69 enrolled patients, 33 and 36 patients were treated or not by EPO, respectively. Among them, 42% had a favorable neurological outcome in both groups. EPO did not affect the transcriptomic response at Day-0 and 1 after OHCA. In contrast, 76 transcripts differed at Day-0 between patients with unfavorable vs favorable neurological outcome. This signature persisted at Day-1 after OHCA. Functional enrichment analysis revealed a down-regulation of adaptive immunity with concomitant up-regulation of innate immunity and inflammation in patients with unfavorable *vs* favorable neurological outcome. The transcription of many genes of the HLA family was decreased in patients with unfavorable *vs* favorable neurological outcome. Concomitantly, neutrophil activation and inflammation were observed. Up-stream regulators analysis showed the implication of numerous factors involved in cell cycle and damages. A logistic regression including a set of genes allowed a reliable prediction of the clinical outcomes (specificity = 88%; Hit Rate = 83%).

Conclusions: A transcriptomic signature involving a counterbalance between adaptive and innate immune responses is able to predict neurological outcome very early after hospital admission after OHCA. This deserves confirmation in a larger population.

Keywords: Cardiac arrest, Cardiopulmonary resuscitation, Prognostication, Transcriptomics, Innate immunity, Inflammation

Introduction

Out-of-hospital cardiac arrest (OHCA) is a major cause of mortality in western countries. After resuscitation and return of spontaneous circulation, neurological, cardiovascular, and multi-organ failures contribute to a "post-cardiac arrest syndrome" that compromises the outcome. Regarding several aspects, this syndrome is considered as a "sepsis-like response".[1]

Using current clinical and biological methods, it is extremely difficult to establish an early and accurate prognosis in this situation. In resuscitated patients with an uncertain outcome, the most recent guidelines specify that clinicians should consider prolonged observation.[2,3] Use of molecular biomarkers is now also proposed to improve prognostication, using micro RNA analyses.[4–7] For instance, higher levels of miR-124-3p were significantly associated with lower survival.[8] However, the use of a single biomarker does not provide a sufficient predictive value and still needs to be combined with other clinical and biological markers.[9]

Our goal was thus to identify a specific signature using a systemic screening evaluation of the whole transcriptome, allowing a deep evaluation of the sepsis-like, inflammatory and blood signaling responses very early after OHCA, *i.e.*, as soon as possible after hospital admission. An exploratory study in 22 patients previously demonstrated that transcriptome analysis could be relevant after OHCA but this was only evaluated after 2 days following resuscitation.[10] Here, we had the opportunity to address the transcriptome since hospital admission in an ancillary study from the large-scale EPO-ACR 02 trial, that failed to demonstrate a clinical effect of erythropoietin (EPO) on survival and neurological outcomes after out-of-hospital cardiac arrest.[11] We compared transcriptomic profiles according to the cerebral performance category (CPC) of the patients at the end of the study. Differential analysis and functional enrichment analysis were performed between patients with unfavorable *versus* favorable outcome, with the purpose to determine the differentially expressed genes, over-represented canonical pathways, and upstream regulators after cardiac arrest in patients. We then identified a gene signature that could predict the neurological outcome from whole blood very early after OHCA. However, this study should be considered as hypothesis generating as we included a limited number of patients.

Methods

General design of the study

The present study was an ancillary study from the EPO-ACR-02 trial (High Dose of Erythropoietin Analogue After Cardiac Arrest), which was a multicenter, phase 3, randomized, single-blind, controlled trial that evaluated the safety and efficacy of a high dose of EPO in patients resuscitated from a cardiac arrest. The designs and results of this principal study were recently published.[11] Briefly, in the intervention group, patients received the first dose of EPO (40,000 units i.v.) as soon as possible after resuscitation. It was followed by 4 injections every 12 h during the first 48 h. In the control group, patients received standard care without any EPO treatment. Neurological performance was assessed at Day-60, according to the cerebral performance category (CPC) scale.[11] We considered favorable and unfavorable neurological outcomes as CPC values ≤ 2 and >2, respectively.

Gene expression profiling

Blood samples were drawn in patients as soon as possible after hospital admission (Day-0) as well as 24 h (Day-1) and 72 h (Day-3) after cardiac arrest. Samples were stored at $-80\,^{\circ}C$ before transcriptomic analyses using Illumina Human HT-12V4 BeadChips that targets 47,323 probes corresponding to 34,694 genes, as described in Supplemental material.

Statistical analyses

Epidemiological and clinical variables were expressed as mean $\pm$ SD or numbers and percentages for continuous and categorical parameters, respectively. Continuous parameters were compared between patients treated by EPO *vs* control and between patients with CPC1-2 *vs* CPC>2 at Day 60 using a two-way analysis of variance. Categorical variables were compared using a Chi-square test. Significant differences were determined by p-values < 0.05.

The differential analysis of the transcriptome consisted in identifying genes differentially expressed between patients treated or not by EPO and/or with CPC1-2 *vs* CPC>2 at Day 60. We also compared transcriptome within different categories of patients according to epidemiological or resuscitation parameters using t-test

analyses. Genes with a fold-change > 1.5 and corrected p-value < 0.05 were considered as differentially expressed as compared to control conditions. Such a high fold-change threshold provides very robust results, despite the rather low number of patients. Functional enrichment analysis was then made using the QIAGEN Ingenuity® Pathways Analysis™ software (www.qiagen.com/ingenuity) using a fold-change > 1.3 and a p-value < 10^{-3}. Indeed, functional enrichment generates hypotheses using wider gene profiling, requiring the use of lower fold-change. Analyses were performed using R (http://www.r-project.org) and Bioconductor (http://www.bioconductor.org). The logistic regression analyses were also performed using R.

Predictive models and logistic regression

A signature of genes that were commonly up- or down-regulated in patients with favorable *vs* unfavorable outcome was derived. The ability of this gene signature to predict the neurological outcome was evaluated by logistic regression. Several regressions were tested in order to determine the best combination regarding specificity, hit rate, and area under the curve (AUC) of the receiving operating curves (ROC).

To compare the prognostic value of the "transcriptomics-based" model to usual clinical parameters, we performed a regression analysis with clinical end-points, including age, low-flow time, no-flow time, initial rhythm during cardiac arrest and epinephrine dosages.

Results

Patients and samples characteristics

As shown in Table 1, we enrolled 69 patients, *i.e.*, 33 and 36 patients treated by EPO or not, respectively. No difference was observed regarding epidemiological characteristics and resuscitation management between these two groups, except for a higher rate of post-cardiac arrest coronary intervention in patients treated by EPO *vs* control. Nevertheless, the neurological outcome was not different since 42% of the patients achieved CPC1-2 at Day 60 in both groups (*i.e.*, 14/33 and 15/36 patients, respectively). The majority of patients with CPC1-2 belonged to the CPC1 category and only 2 patients belonged to the CPC2 category (*i.e.*, 1 in each EPO and control group). Conversely, a large majority of patients with poor neurological (CPC>2) outcome belonged to the CPC5 category (death) and only 2 patients belonged to the CPC3 category (*i.e.*, 1 in each EPO and control group). No patient was in the CPC4 category. As compared to patients with CPC>2, patients with favorable outcome (CPC1-2), were younger, with a shorter low-flow time and less epinephrine used during resuscitation.

Among these 69 patients, 181 blood samples were collected from the 207 expected samples (3 samples planned for each patient). Missing samples were related to early death (n = 14) or lack of sampling in surviving patients (n = 12). Among the 181 available samples, 7 samples were excluded for technical reasons after quality control. We ultimately analyzed transcriptomic data from 174 blood samples (64, 62 and 52 at Day-0, 1 and 3, respectively). Sample repartition among the different conditions and time of collection after cardiac arrest are shown in Supplemental Table 1.

Effect of EPO on the transcriptome after cardiac arrest

As shown in Fig. 1A, only 1 and 4 genes were differentially expressed at Day-0 and Day-1 between patients treated with EPO or not, respectively. This number significantly increased at Day-3 (141 genes), with a homogeneous modification of the transcriptome among most patients (Fig. 1B). As expected and illustrated by Fig. 1C, functional enrichment revealed an activation of heme biosynthesis pathways at Day-3 in patients treated by EPO.

Effect of epidemiological and clinical parameters on the transcriptome after cardiac arrest

As illustrated in Fig. 2A, we then assessed the effect of epidemiological and resuscitation parameters on gene transcriptome, as compared

Table 1 – Epidemiological and clinical findings.

Mean ± SD, N or %	Control		EPO		Pooled CPC1-2	Pooled CPC>2
	CPC1-2	CPC>2	CPC1-2	CPC>2		
Number of patients and % among group	15 (42%)	21 (58%)	14 (42%)	19 (58%)	29 (42%)	40 (58%)
Age (years)	53 ± 16	62 ± 12*	55 ± 16	60 ± 11*	54 ± 16	61 ± 12*
Gender (male/female)	12/3	16/5	11/3	17/2	23/6	33/7
Initial shockable rhythm (N [%])	9 (60%)	10[a] (50%)	11 (79%)	9 (47%)	20 (69%)	20[a] (51%)
Epinephrine dose (mg)	0.33 ± 0.90	4.00 ± 4.05*	1.57 ± 2.93	3.00 ± 2.93*	0.63 ± 2.19	3.53 ± 3.55*
No-flow time(min)	2.9 ± 2.9	5.8 ± 5.8	2.9 ± 5.8	4.3 ± 4.3	2.9 ± 4.3	4.3 ± 4.3
Low-flow time (min)	14.4 ± 8.6	27.4 ± 15.8*	14.4 ± 11.5	25.9 ± 14.4*	14.4 ± 10.1	27.4 ± 14.4*
Post-cardiac arrest PCI (N [%])	4 (27%)	7 (33%)	9 (64%)†	12 (63%)†	13 (45%)	19 (48%)
Thrombotic events (N [%])	1 (7%)	2 (10%)	2 (14%)	3 (16%)	3 (10%)	5 (13%)
Cardiogenic shock status (N [%])	3 (20%)	7 (33%)	5 (36%)	10 (53%)	8 (28%)	17 (42%)
Body temperature at H24 (°C)	34.0 ± 1.6	33.9 ± 1.7	33.0 ± 0.8	33.8 ± 1.3	33.3 ± 1.3	33.9 ± 1.5
Body temperature at H48 (°C)	37.5 ± 0.9	37.1 ± 1.7	37.5 ± 1.0	37.3 ± 1.2	37.5 ± 0.9	37.3 ± 1.4

Data are expressed as number, percentage or mean ± SD. Percentages were calculated within each sub-group (*i.e.*, column), except for the total number of patients with CPC 1–2 or CPC>2 which includes the percentage of patients among the whole control and EPO groups. PCI, percutaneous intervention.

[a] Unknown for 1 patient.

* $p < 0.05$ *vs* corresponding CPC1-2.

† $p < 0.05$ *vs* corresponding control.

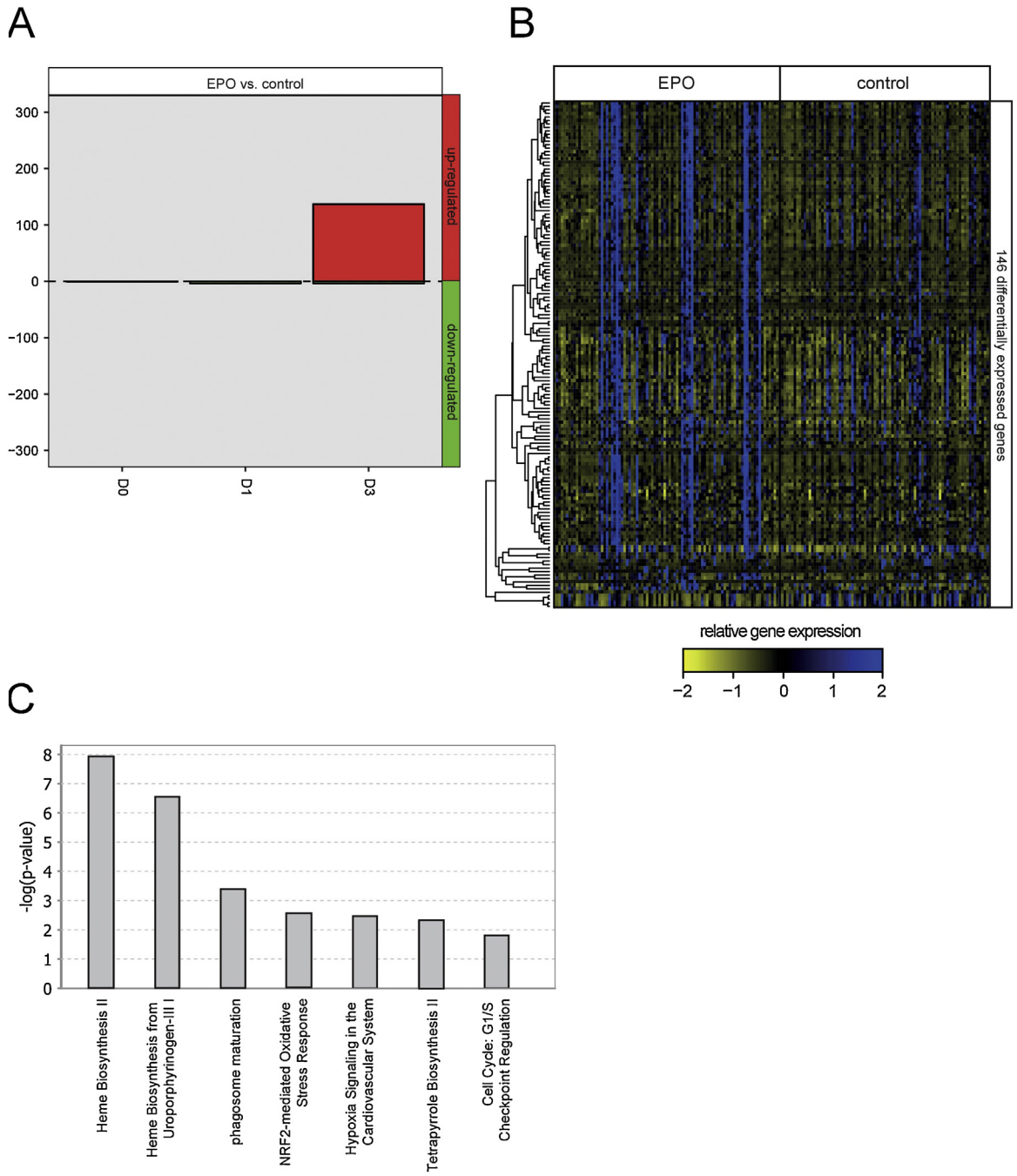


Fig. 1 – Effect of erythropoietin (EPO) on the transcriptome after cardiac arrest.
(A) Bar chart representation showing the number of probes differentially expressed between EPO and control samples at Days 0, 1 and 3. Up-regulated probes are indicated in red while down-regulated probes are indicated in green.
(B) Heatmap showing the relative expressions for genes differentially expressed in at least one timepoint. Probes were organized based on their expression profiles using a hierarchical clustering.
(C) Bar chart representation showing the p-values, displayed as $-\log_{10}$, of pathways found to be significantly over-represented at Day-3 in patients treated by EPO *vs* controls. Functional enrichment revealed an activation of heme biosynthesis pathways at Day-3 in patients treated by EPO (*e.g.*, $p = 10^{-7.9}$, $10^{-6.5}$, and $10^{-2.3}$ for heme biosynthesis II, heme biosynthesis from uroporphyrinogen III-1, and Tetrapyrrole Biosynthesis II pathways, respectively). We also observed an activation of other pathways linked to EPO signaling such as phagosome maturation, NRF2-mediated Oxidative Stress Response or hypoxia signalling in the cardiovascular system ($p = 10^{-3.4}$, $10^{-2.5}$, and $10^{-2.5}$, respectively).

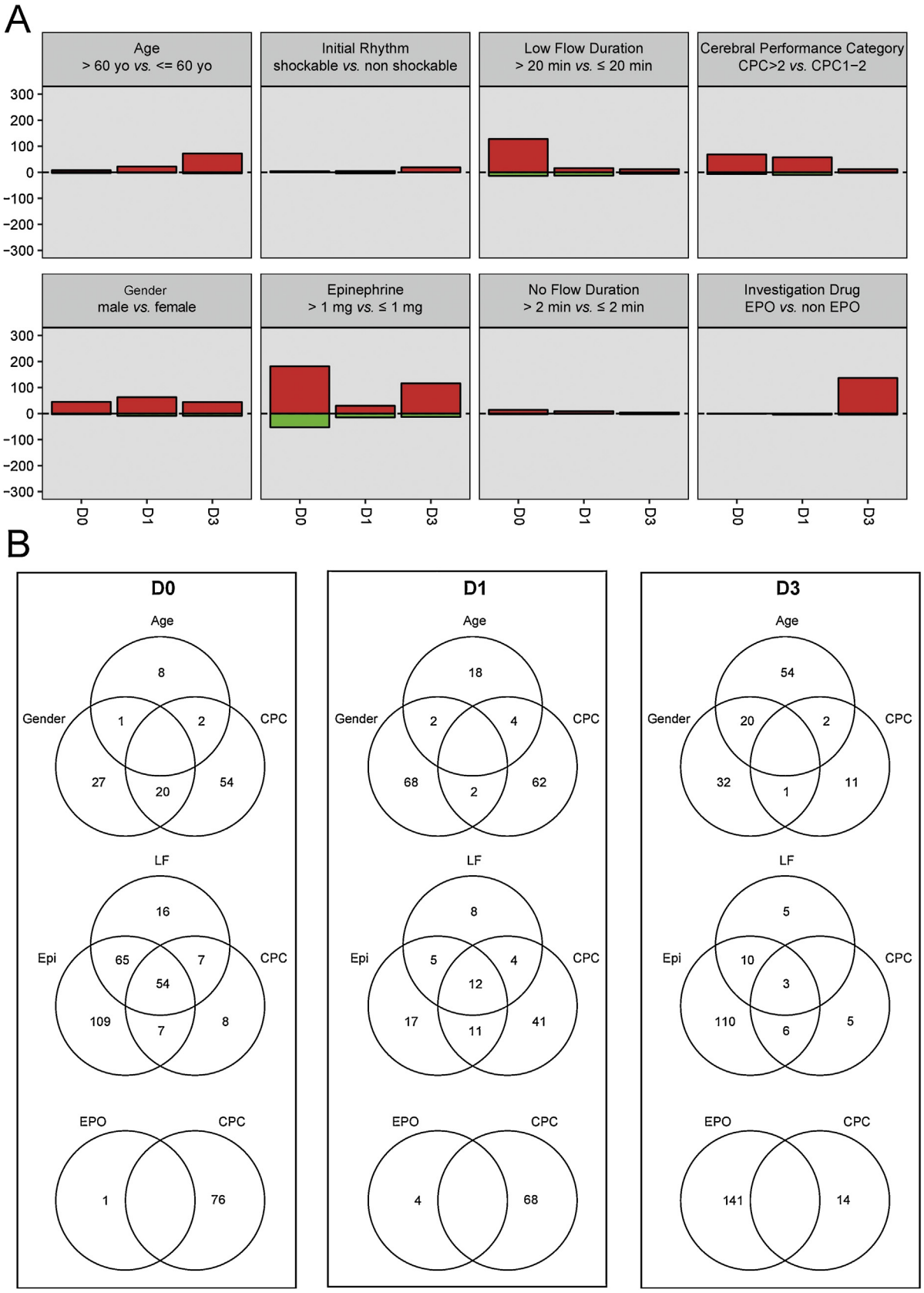


Fig. 2 – Effect of epidemiological, resuscitation and outcome parameters on the blood transcriptome evaluated at Day-0, Day-1 and Day-3 after cardiac arrest.

(A) Bar chart representation showing the number of probes differentially expressed between patients depending upon the age, gender, initial rhythm of cardiac arrest, epinephrine (Epi) dose before resuscitation, low-flow (LF) duration, no-

to EPO proper effect. Gene expressions mostly differ regarding gender (*e.g.*, 48 genes at Day-0), low-flow duration (142 genes at Day-0), ultimate CPC category (CPC>2 *vs* CPC1-2; 76 genes at Day-0) and epinephrine dose (>1 mg *vs* ≤1 mg; 235 genes at Day-0). Among the modified transcripts, no gene was commonly modified between age category or gender and CPC (Fig. 2B). Similarly, no gene was commonly modified between CPC and EPO categories. This demonstrates that the transcriptomic signature marking CPC was not biased by age, gender or EPO treatment. Conversely, many genes were commonly modified among CPC categories and low-flow duration or epinephrine dose. This was expected as the latter parameters are well-known predictors of neurological outcome. For instance, 54 and 12 genes were commonly modified by CPC categories, low-flow duration or epinephrine dose at Day-0 and Day-1 after cardiac arrest. Supplemental Fig. 1 lists these common genes.

Low-flow duration and epinephrine dose modified many other genes beyond the CPC signature, demonstrating a transcriptomic response non-specific of the ultimate outcome. For instance, only 43 and 26% of the genes modified by low-flow duration and epinephrine dose at Day-0 were common with those modified in CPC>2 *vs* CPC1-2 patients, respectively. Therefore, further analyses were focused on the proper transcriptomic signature of CPC category at Day-0 and Day-1, which was the most relevant for a prognostication purpose. We also focused on Day-0 and Day-1 as 15 samples were missing at Day-3, due to the death of patients or technical considerations, which probably flawed the results at this time point and explains the decreased number of differentially expressed genes.

Transcriptomic differences in patients with favorable vs unfavorable outcome

As illustrated in Fig. 3A (Day-0) and 3B (Day-1), we then examined the top up- and down-differentially expressed genes in patients with CPC>2 *vs* CPC1-2. Among them, we found a majority of genes involved in inflammation, innate immunity and cell migration. We also found several genes involved in cell cycle regulation and heat shock proteins.

In order to assess the most relevant and time-independent genes differing in patients with CPC>2 *vs* CPC1-2, we determined the common ones at Day-0 and Day-1 (Fig. 3C). As illustrated in Fig. 3D, we identified 17 genes with, again, a majority is known to be at least in part linked to immunity and inflammatory response. We also found genes related to cell cycle or response to DNA damages.

Functional enrichment analyses of transcriptomic data

We pursued our analysis by a functional enrichment of the gene list generated at Day-0 and Day-1. Fig. 4A illustrates the canonical pathways found to be over-represented between patients with unfavorable *vs* favorable outcome. Briefly, antigen presentation pathways, OX40, or Cdc42 signaling were significantly down-regulated in patients with CPC>2 compared to CPC1-2. In contrast, interleukin-6 signaling and toll-like receptor (TLR) signaling were significantly up-regulated and enriched in patients with CPC>2 *vs* CPC1-2. Communication between innate and adaptive immune cells and crosstalk between dendritic cells and natural killer cells pathways followed a biphasic pattern of alterations with a significant up-regulation at Day-0 and subsequent a down-regulation at Day-1 in patients with CPC>2 *vs* CPC1-2.

As illustrated in Fig. 4B, upstream regulators analysis further predicted the implication of many factors that could be linked to inflammation (*e.g.*, CCAAT/enhancer-binding protein alpha [CEBPA], signal transducer and activator of transcription 3 [STAT3], high mobility group box 1 [HMGB1]) or cell cycle regulation and nuclear damage signaling.

In order to further explore the putative pathways linked to the ultimate outcome, we conducted a further analysis using only the genes that were commonly modified at Day-0 and Day-1 in patients with CPC>2 *vs* CPC1-2. This includes 94 genes using a 1.3 fold change (*vs* 17 genes in the previous analysis illustrated by Fig. 3 with a 1.5 fold change). As illustrated in Fig. 4C, the canonical pathways linked to the ultimate patient's outcome were then all related to adaptive immune responses including antigen presentation, OX40 signaling pathways, Th1/Th2 activation pathway or Cdc42 signaling.

Prognostication value of the transcriptomic signature

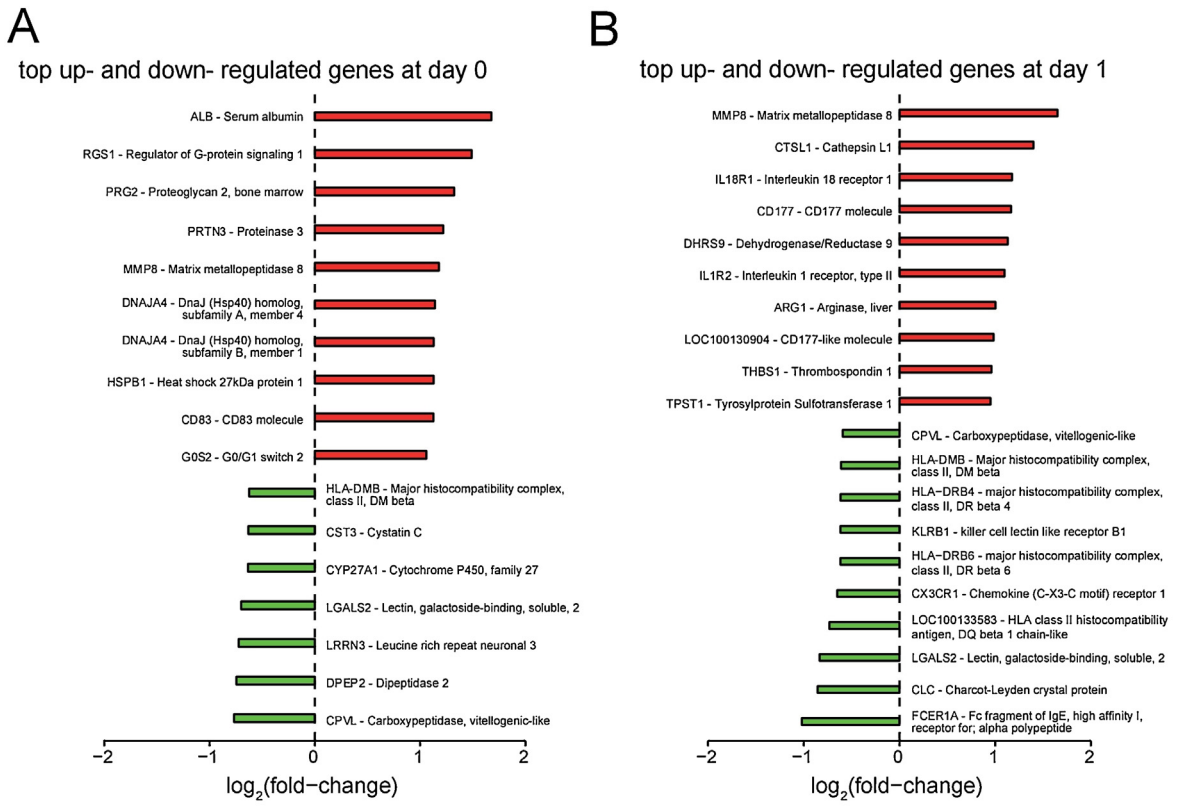
Using the wide list of 94 genes commonly modified at Day-0 and Day-1, we determined whether a logistic regression using a combination of genes could better predict the ultimate outcome at Day 60 (CPC>2 *vs* CPC1-2). In order to be more discriminant for a prognostication purpose, this model was tested on the entire bank of blood samples, *i.e.*, in blood samples obtained at Day-0, Day-1 but also Day-3. The general linear model with the best prognostication value was obtained with a combination of 38 genes (Fig. 5A). Using this combination of genes, we generated a general linear model providing a high specificity of 88%, a hit rate of 83%, and a ROC AUC of 0.94 (Fig. 5B).

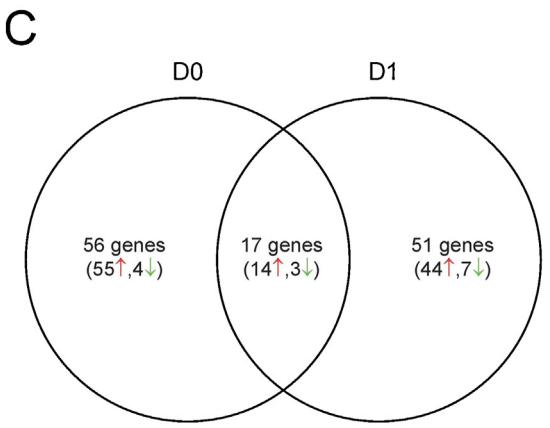
As a reference approach, we then tested a logistic regression using conventional clinical and epidemiological parameters, *i.e.*, age, epinephrine dose, low-flow time, no-flow time and initial rhythm before resumption of spontaneous circulation (Table 1). As illustrated in Fig. 5C, the combination of these clinical and epidemiological parameters predicts outcome with a specificity, hit rate, and AUC of ROC lower than the transcriptomics model.

Discussion

Using a screening analysis of the whole transcriptome, we deciphered the systemic response following OHCA, generating a new hypothesis for patient prognostication after out-of-hospital cardiac arrest. We used a population treated or not by EPO, which did not affect outcome. Furthermore EPO did not modify the early transcriptomic response to OHCA but only at Day-3 after cardiac arrest. Accordingly, we focused our analyses on the transcriptomic response of the patients at Day-0 and Day-1. Since the first blood sample drawn after hospital admission, more than 300 genes were differentially expressed between patients with unfavorable (CPC>2) *vs* favorable neurological

flow-duration, cerebral performance category (CPC) at day 60 after cardiac arrest, and putative treatment (EPO). Up-regulated probes are indicated in red, while down- regulated probes are indicated in green.
(B) Venn diagram showing the number of differentially expressed genes in patients with CPC>2 *vs* CPC1-2 and other above-listed investigated parameters.





D

Gene Symbol	D0	D1	Gene Name
MMP8	2.26	3.14	Matrix metallopeptidase 8
CTSL1	1.85	2.64	Cathepsin L1
CD177	1.93	2.24	CD177 molecule
DDIT4	1.80	1.84	DNA-damage-inducible transcript 4
SMIM1	1.78	1.85	Small integral membrane protein 1
CLEC1B	1.68	1.80	C-Type Lectin Domain Family 1 Member B
GPR84	1.90	1.57	G protein-coupled receptor 84
RETN	1.83	1.61	adipose tissue-specific secretory factor
CDC20	1.68	1.71	cell-division cycle protein 20
ANKRD22	1.58	1.71	Ankyrin Repeat Domain 22
SAMSN1	1.53	1.74	SAM domain-containing protein SAMSN-1
CEACAM1	1.52	1.75	Carcinoembryonic antigen-related cell adhesion molecule 1
STOM	1.51	1.55	Stomatin
TNFAIP6	1.50	1.54	TNF Alpha Induced Protein 6
HLA-DMB	-1.54	-1.53	Major histocompatibility complex, class II, DM beta
CPVL	-1.70	-1.51	carboxypeptidase, vitellogenic-like
LGALS2	-1.62	-1.78	lectin, galactoside-binding, soluble, 2

Fig. 3 – Differentially expressed genes in patients with an ultimate cerebral performance category (CPC)>2 *vs* CPC.1-2. Down-regulated genes are indicated in green, while up-regulated genes are indicated in red.
(A and B) List of the top up- and down-regulated genes with their associated fold-changes at Day-0 and Day-1 after cardiac arrest in patients with CPC>2 *vs* CPC1-2, respectively. A majority of genes are involved in inflammation, innate immunity and cell migration, such as matrix metalloproteinase-8 (MMP-8, an enzyme stored in secondary granules within neutrophils), several genes encoding for major histocompatibility complex (HLA-DMB, HLA-DRB4, HLA-DRB6), CD177 (cell surface glycoprotein playing a role in neutrophil activation), CD83 (member of the immunoglobulin superfamily receptors, involved in immune response), IL1R2 (Interleukin [IL]-1 receptor, type II), IL18R1 (Interleukin [IL]-18 receptor 1), PRTN3 (Proteinase 3, that could play a role in in neutrophil migration), RGS1 (regulator of G-protein signaling 1), PRG2 (Proteoglycan 2, Pro Eosinophil Major Basic Protein) or CX3CR1 (CX3C chemokine receptor 1, a transmembrane protein and chemokine involved in the adhesion and migration of leukocytes). We also observe several genes involved in cell cycle regulation and heat shock proteins, such as DNAJ (DnaJ [Hsp40] homolog subfamily, members 1 and 4), HSPB1 (Heat shock 27 kDa protein 1) or G0S2-G0/G1 switch 2. Finally, we observed an up-regulation of cathepsin L1 (CTSL1), a lysosomal cysteine protease that plays a major role in intracellular protein

outcome (CPC≤2), *i.e.*, only few hours after resuscitation. Using a combination of genes, we were able to predict the outcome with a good specificity, hit rate and AUC of the ROC curves. This is one the first biomarker approach providing promising results for prognostication since hospital admission. However, results should be interpreted with caution according to the small number of patients.

To our knowledge, this is the first wide genome-wide analysis of the very early and global response following resuscitation in OHCA patients. A transcriptomic signature was observed in patients with unfavorable outcome involving both adaptative immunity, through antigen presentation, and innate immunity and inflammation transcripts. Even if several clinical and non-clinical prognostic markers are already usable, there is still a need for very early prognostication tools. Here we observed that the transcriptomic signature may provide information on hospital admission.

In patients with an unfavorable outcome, a complex balance occurred in the immune response through up-regulation of innate immunity and down-regulation of antigen presentation processes. The latter event was striking with a significant alteration at both Day-0 and Day-1 in patients with CPC>2 *vs* CPC≤2. This was supported by the functional enrichment analysis as well as many top up- and top down-transcripts related to the HLA family (HLA-DMB, HLA-DRB3, HLA-DRB4, HLA-DRB6). This could suggest a deficiency of antigen-presentation in patients with unfavorable outcome.

Beyond the down-regulation of adaptative immunity in patients with an unfavorable outcome, we observed an activation of innate immunity and inflammation in this category of patients. For example, neutrophil activation is shown by metalloproteinase-8 (MMP-8) and CD177 upregulation in patients with the unfavorable *vs* favorable outcome, suggesting a deleterious effect of neutrophil activation. Proteinase 3 (PRTN3) and MMP8 are well-known protease genes markers of sepsis, showing a clear engagement of neutrophils response.[12] We also observed a significant activation of several interleukins and TLRs, as well as an up-regulation of CD83, Regulator of G-protein signaling 1 (RGS1) and Proteinase 3 (PRTN3), which play a key role in inflammation in patients with poor outcome. Among these genes, regulator of G-protein signaling 1 (RGS1) has an important role in the regulation of B and T lymphocytes and macrophages trafficking and functions. RGS1 has been involved in macrophage-mediated vascular inflammation in atherosclerotic plaques.[13] Genome-wide association studies (GWAS) also identified a link between polymorphic variants of RGS1 and chronic inflammatory diseases in humans, including celiac disease, multiple sclerosis, and type I diabetes.[14–16]

Importantly, the apparent contradiction between the down-regulation of antigen presentation pathways and the activation of innate immunity could be explained by the biphasic pattern of evolution of the crosstalk between dendritic cells and natural killer cells and the communication between innate and adaptive immune cells. This supports that a dysfunctional immune response occurs in patients with an unfavorable outcome, with an inappropriate balance between innate and adaptative responses through antigen presentation. In other words, a strong innate immunity and inflammatory response may occur in these patients, with no ability to activate a subsequent and beneficial adaptative response. Such a hypothesis could represent a paradigm shift as compared to the concept of sepsis-like response after OHCA, which should be revisited in the light of a more specific activation of some immune pathways. Obviously, these results are only hypothesis-generating.

The activation of innate immunity and inflammation is also consistent with up-stream regulators analysis which identified a role of the HMGB1 pathway. This is a well established damage-associated molecular pattern (DAMPs) that could activate immunity and have multiple functions in the regulation of immunity and inflammation. This is in agreement with the danger-model of immunity which was deciphered during stroke[17] or sepsis,[18] as well as the differential expression of several genes related to DNA damages. Beyond the immunity alteration provoked by danger signaling, the up-stream regulators analysis also evidenced the implication of many other factors. For instance, carcinoembryonic antigen-related cell adhesion molecule 1 (CEBPA) is known to increase macrophage inflammatory protein (MIP)-2 production from mast cells upon bacterial stimulation.[19] Likewise, Forkhead box O1 (FOXO1) affects several critical aspects of neutrophil functions including mobilization of neutrophils from the bone marrow to the vasculature, recruitment of neutrophils and clearance of bacteria. Furthermore, bacteria-induced nuclear localization of FOXO1 is dependent upon TLR-2 and/or TLR4,[20] that could be related to the sepsis-like syndrome observed in OHCA. The involvement of estrogen receptor 1 (ESR1), as a direct regulator of the innate and adaptive immune system was also observed and could participate in

catabolism, as well as other genes with still questionable putative function in the setting of cardiac arrest (*e.g.*, albumin).

(C) Venn diagram showing the number of differentially expressed genes found at Day-0 and Day-1 in patients with CPC > 2 *vs* CPC1-2.

(D)List of the top up- and down-regulated genes commonly modified at Day-0 and Day-1 in patients with an ultimate cerebral performance category (CPC)>2 *vs* CPC 1-2 with their associated fold-changes. A majority of genes are known to be at least in part linked to immunity and inflammatory response, *e.g.*, MMP8, CD177, CLEC1B (C-type lectin-like receptor, which is expressed in myeloid cells and natural killer cells), GPR84 (G Protein-Coupled Receptor 84, also known as Inflammation-Related G Protein-Coupled Receptor EX33), RETN (adipose tissue-specific secretory factor, which promotes chemotaxis in myeloid cells), SAMSN1 (SAM domain-containing protein, a negative regulator of B-cell activation), CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1, which belongs to the immunoglobulin superfamily), TNFAIP6 (tumor necrosis factor alpha-induced protein 6), HLA-DMB, or CPVL (carboxypeptidase vitellogenic-like protein, which may participate to the inflammatory protease cascade). Some genes were also related to cell cycle or response to DNA damages such as DDTI4 (DNA-damage-inducible transcript 4), CDC20 (cell-division-cycle protein 20, which interacts with several other proteins at multiple points in the cell cycle). The putative function of the 5 last identified genes is still more challenging to identify in the cardiac arrest setting, *i.e.*, CTSL1, SMIM1 1 (Small Integral Membrane Protein 1), ANKRD22 (Ankyrin Repeat Domain 22), STOM (Stomatin, a highly conserved family of integral membrane proteins), LGALS2 (lectin galactoside-binding soluble, also known as Galectin 2).

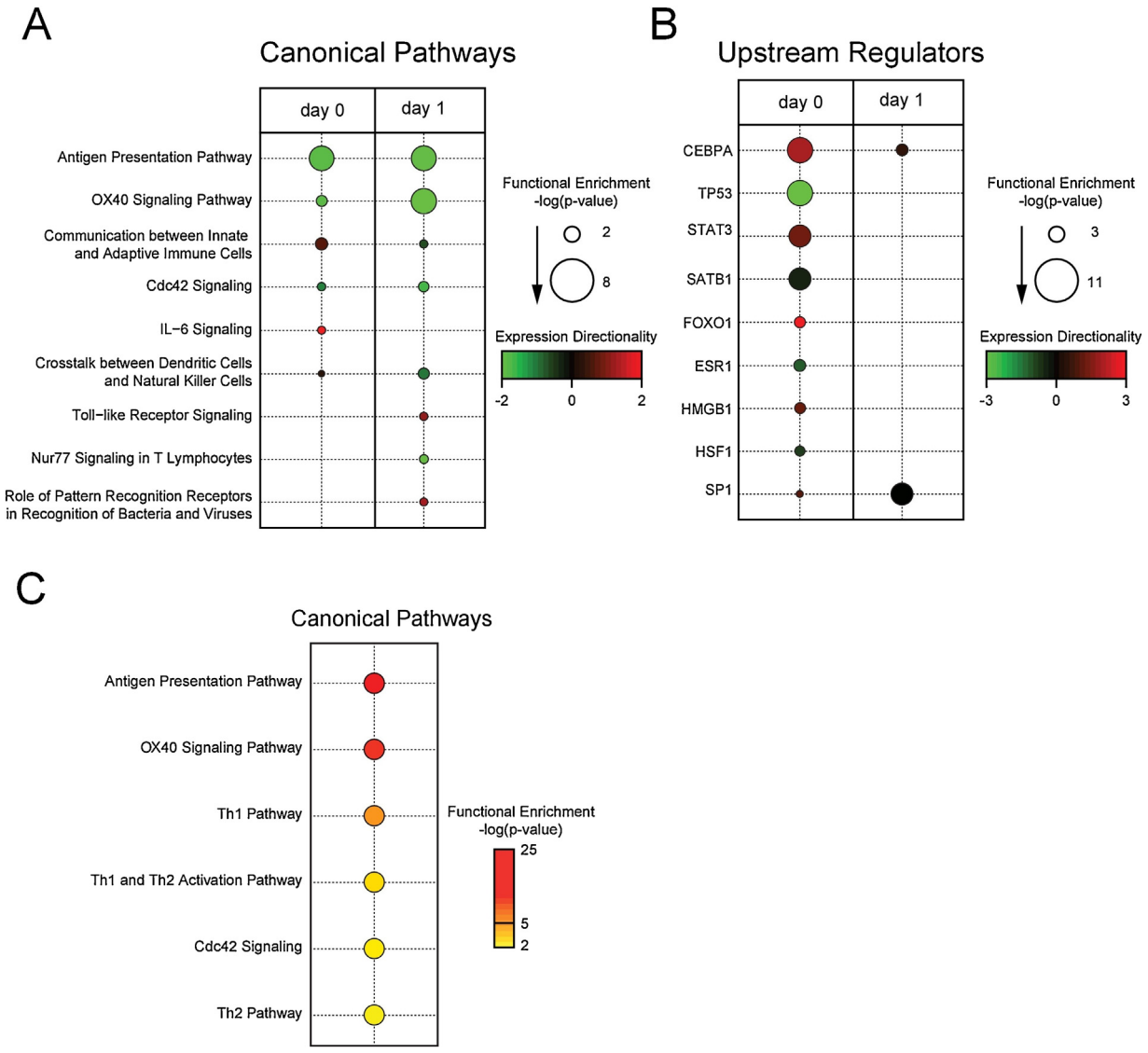


Fig. 4 – Functional enrichment of the transcriptomic signature predicting patient outcomes after cardiac arrest in patients with cerebral performance category (CPC)>2 *vs* CPC1-2.

(A) Functional enrichment analysis showing the canonical pathways found to be over-represented at Day-0 and Day-1 in patients with CPC>2 *vs* CPC1-2. Some pathways were significantly down-regulated at Day-0 and/or Day-1, such as antigen presentation pathways, OX40, Cdc42 signaling and Nur77 Signaling (*e.g.*, $p = 10^{-7.5}$, $10^{-5.3}$, $10^{-4.2}$, $10^{-4.2}$ at Day-0 and $p = 10^{-4.4}$ at Day-1 respectively). Oher pathways were significantly up-regulated, such as interleukin-6 signaling ($p = 10^{-4.1}$ at Day-0), toll-like receptor (TLR) signaling ($p = 10^{-4.2}$ at Day-1), and pattern recognition receptors in recognition of bacteria and viruses ($p = 10^{-4.0}$ at Day-1). Communication between innate and adaptive immune cells and crosstalk between dendritic cells and natural killer cells pathways followed a biphasic pattern of alterations with a significant up-regulation at Day-0 and subsequent a down-regulation at Day-1.

(B) Functional enrichment analyses of upstream regulators found to be over-represented in gene signatures at Day-0 and Day-1 in patients with CPC>2 *vs* CPC1-2. The p-value, associated with each pathway, is represented $-\log_{(p\text{-value})}$ using a gradient color scale. For instance, CCAAT/enhancer-binding protein alpha (CEBPA) and Sp1 transcription factor (SP1) were significantly activated at both Day-0 and Day-1 (*e.g.*, $p = 10^{-10.1}$ and $p = 10^{-8.3}$ at Day-0). Other regulators were significantly modified at only Day-0, with either down-regulation (TP53 — tumor protein 53, ESR1 — Estrogen Receptor 1, and HSF1 — Heat Shock Transcription Factor 1) or up-regulation (SATB1 — Special AT-rich sequence binding protein 1, STAT3 — signal transducer and activator of transcription 3, FOXO1 — Forkhead box O1, HMGB1 — high mobility group box 1). Many factors could be linked to inflammation (CEBPA, STAT3, HMGB1) or cell cycle regulation and nuclear damage signaling (TP53, FOXO1, SATB1, HMGB1, SP1).

(C) Functional enrichment analysis showing the pathways found to be over-represented among the common genes at Day-0 and Day-1 in patients with CPC>2 *vs* CPC1-2. The p-value is represented using a color-gradient scale.

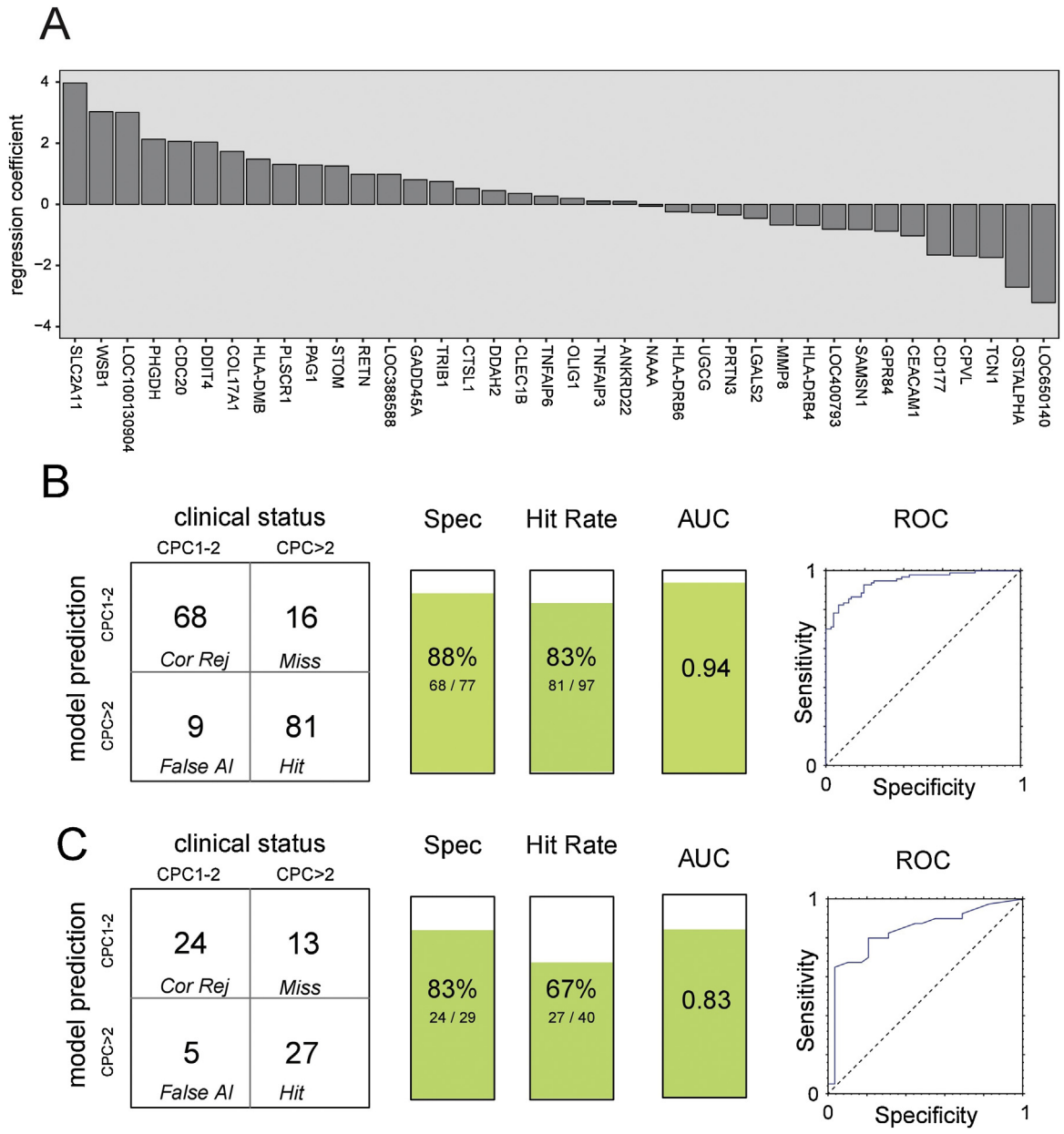


Fig. 5 – Logistic model based on the transcriptomic signature (Panels A and B) or clinical and epidemiological findings (Panel C) to predict the outcome of the patients with cerebral performance category (CPC)>2 *vs* CPC.1-2.

(A) Coefficients associated with each gene in the logistic regression analysis. p-values associated with each coefficient are indicated using a color gradient scale. The regression analysis was based on 38 genes, including a majority of genes already presented in previous analyses, such as CDC20, DDIT4, HLA-DMB, STOM, RETN, CLEC1B, TNFAIP6, ANKRD22, HLA-DRB6, PRTN3, LGALS2, MMP8, HLA-DRB4, SAMSN1, GPR84, CD177 and CPVL (see Legend of Figs. 3 and 4). Additional genes were also listed here, *e.g.*, SLC2111 (Solute Carrier Family 2 Member 11), COL17A1 (collagen type XVII alpha 1 chain), PHGDH (phosphoglycerate dehydrogenase), OST-ALPHA (organic solute transporter subunit alpha) or TCN1 (transcobalamin 1).

(B) Summary of the ability of the transcriptomic logistic model to predict the outcome of the patients, including the truth/decision table, specificity, hit rate, AUC, and the ROC curve.

(C) Summary of the ability of the logistic model based on clinical and epidemiological parameters to predict the outcome of the patients, including the same parameters.

gender differences.[21] These findings allow generating hypothesis regarding the putative damage (DNA damage, apoptosis) or repairing process (cell lineage, maturation and hematopoïesis) occurring after cardiac arrest.

This study presents several limitations. For instance, we might further improve the specificity of the transcriptomic signature through a deeper analysis taking into account the cause of death, *e.g.*, hemodynamic or cerebral failure. A replication of the results will also be required prior to a wide implementation of the transcriptomic signature as prognostic tool in the clinical arena. We should also keep in mind that the results were only obtained using a limited number of patients.

Conclusion

In conclusion, this wide analysis of the transcriptome demonstrates a counterbalance between adaptive and innate immune response after cardiac arrest. A clear transcriptomic signature was observed and allows to predict favorable outcome very early after cardiac arrest since hospital admission. This generates new hypotheses and provides promising perspectives for the prognostication after OHCA, and more widely in cardiovascular diseases. A combinatory approach including transcriptomic signatures could be the key for the future management of OHCA.[22–25] In the future general practice, one would speculate that dedicated micro-arrays or next generation sequencing (NGS) approaches could provide rapid prognostic tools at admission, as also expected for trauma brain injury or stroke.[26,27] However, even if providing important information on outcome, further prospective validation studies on larger samples are required that may improve the predictive performance of the transcriptomic model and its use for individual prognostication.

Conflict of interest

The authors do not disclose conflict of interest.

Sources of funding

This study was supported by the AREM CAR (Association; Créteil, France) and Agence Nationale pour la Recherche (COOLIVENT Grant). Nicolas Tchitchek was supported by fellowships from the ANRS (France Recherche Nord & Sud Sida-hiv Hépatites).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.resuscitation.2019.03.006.

REFERENCES

1. Adrie C, Adib-Conquy M, Laurent I, et al. Successful cardiopulmonary resuscitation after cardiac arrest as a “sepsis-like” syndrome. Circulation 2002;106:562–8.
2. Sandroni C, Cariou A, Cavallaro F, et al. Prognostication in comatose survivors of cardiac arrest: an advisory statement from the European Resuscitation Council and the European Society of Intensive Care Medicine. Intensive Care Med 2014;40:1816–31.
3. Nolan JP, Soar J, Cariou A, et al. European Resuscitation Council and European Society of Intensive Care Medicine 2015 guidelines for post-resuscitation care. Intensive Care Med 2015;41:2039–56.
4. Stammet P, Goretti E, Vausort M, Zhang L, Wagner DR, Devaux Y. Circulating microRNAs after cardiac arrest. Crit Care Med 2012;40:3209–14.
5. Gilje P, Gidlöf O, Rundgren M, et al. The brain-enriched microRNA miR-124 in plasma predicts neurological outcome after cardiac arrest. Crit Care 2014;18:R40.
6. Devaux Y, Stammet P, Friberg H, et al. MicroRNAs: new biomarkers and therapeutic targets after cardiac arrest? Crit Care 2015;19:54.
7. Wander PL, Enquobahrie DA, Pritchard CC, et al. Circulating microRNAs and sudden cardiac arrest outcomes. Resuscitation 2016;106:96–101.
8. Devaux Y, Dankiewicz J, Salgado-Somoza A, et al. Association of circulating microRNA-124-3p levels with outcomes after out-of-hospital cardiac arrest: a substudy of a randomized clinical trial. JAMA Cardiol 2016;1:305–13.
9. Nolan JP, Soar J, Cariou A, Cronberg T, et al. European Resuscitation Council and European Society of Intensive Care Medicine guidelines for post-resuscitation care 2015. Resuscitation 2015;95:202–22.
10. Eun JW, Yang HD, Kim SH, et al. Identification of novel biomarkers for prediction of neurological prognosis following cardiac arrest. Oncotarget 2017;8:16144–57.
11. Cariou A, Deye N, Vivien B, et al. Early high-dose erythropoietin therapy after out-of-hospital cardiac arrest a multicenter, randomized controlled trial. J Am Coll Cardiol 2016;68.
12. Almansa R, Ortega A, Ávila-Alonso A, et al. Quantification of immune dysregulation by next-generation polymerase chain reaction to improve sepsis diagnosis in surgical patients. Ann Surg 2017;1.
13. Patel J, McNeill E, Douglas G, et al. RGS1 regulates myeloid cell accumulation in atherosclerosis and aortic aneurysm rupture through altered chemokine signalling. Nat Commun 2015;6:6614.
14. Smyth DJ, Plagnol V, Walker NM, et al. Shared and distinct genetic variants in type 1 diabetes and celiac disease. N Engl J Med 2008;359:2767–77.
15. Hunt KA, Zhernakova A, Turner G, et al. Newly identified genetic risk variants for celiac disease related to the immune response. Nat Genet 2008;40:395–402.
16. Johnson BA, Wang J, Taylor EM, et al. Multiple sclerosis susceptibility alleles in African Americans. Genes Immun 2010;11:343–50.
17. Shichita T, Ito M, Morita R, et al. MAFB prevents excess inflammation after ischemic stroke by accelerating clearance of damage signals through MSR1. Nat Med 2017;23:723–32.
18. Lan K-C, Chao S-C, Wu H-Y, et al. Salidroside ameliorates sepsis-induced acute lung injury and mortality via downregulating NF-κB and HMGB1 pathways through the upregulation of SIRT1. Sci Rep 2017;7:12026.
19. Kasakura K, Takahashi K, Itoh T, et al. C/EBPα controls mast cell function. FEBS Lett. 2014;588:4645–53.
20. Dong G, Song L, Tian C, et al. FOXO1 regulates bacteria-induced neutrophil activity. Front Immunol 2017;8:1088.
21. Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. Cell Immunol 2015;294:63–9.
22. Viereck J, Thum T. Circulating noncoding RNAs as biomarkers of cardiovascular disease and injury. Circ Res 2017;120:381–99.
23. Uchida S, Dimmeler S. Long noncoding RNAs in cardiovascular diseases. Circ Res 2015;116:737–50.
24. Thum T, Condorelli G. Long noncoding RNAs and microRNAs in cardiovascular pathophysiology. Circ Res 2015;116:751–62.
25. Annborn M, Nilsson F, Dankiewicz J, et al. The combination of biomarkers for prognostication of long-term outcome in patients treated with mild hypothermia after out-of-hospital cardiac arrest—a pilot study. Ther Hypothermia Temp Manag 2016;6:85–90.
26. Meng Q, Zhuang Y, Ying Z, et al. Traumatic brain injury induces genome-wide transcriptomic, methylomic, and network perturbations in brain and blood predicting neurological disorders. EBioMedicine 2017;16:184–94.
27. Kessler T, Schunkert H. Genetics of recovery after stroke. Circ Res 2019;124:18–20.