Comet assay in evaluating deoxyribonucleic acid damage after out-of-hospital cardiac arrest

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Abstract

Objective: This study aimed to investigate whether out-of-hospital cardiac arrest (OHCA) may induce severe DNA damage measured using comet assay in successfully resuscitated humans and to evaluate a short-term prognostic role.

Methods: In this prospective, controlled, blinded study (1/2013–1/2014), 41 patients (age, 63±14 years) successfully resuscitated from non-traumatic OHCA and 10 healthy controls (age, 53±17 years) were enrolled. DNA damage [double-strand breaks (DSBs) and single-strand breaks (SSBs)] was measured using comet assay in peripheral lymphocytes sampled at admission. Clinical data were recorded (according to Utstein style). A good short-term prognosis was defined as survival for 30 days.

Results: Among the patients, there were 71% (29/41) short-term survivors. After OHCA, DNA damage (DSBs and SSBs) was higher (11.0±7.6% and 0.79±2.41% in tail) among patients than among controls (1.96±1.63% and 0.02±0.03% in tail), and it was more apparent for DSBs ($p<0.001$ and $p=0.085$). There was no difference in the DNA damage between patients with cardiac and non-cardiac etiology, or between survivors and non-survivors. Among Utstein style parameters, ventricular fibrillation, asystole, and early electrical defibrillation influenced DSBs; none of the factors influenced SSBs. Factors influencing survival were SSBs, ventricular fibrillation, length of cardiopulmonary resuscitation by professionals ≤15 min, cardiogenic shock, and postanoxic encephalopathy. In contrast to DSBs [area under the curve (AUC)=0.520], SSBs seem to have a potential in prognostication (AUC=0.639).

Conclusion: This study for the first time demonstrates revelation of DNA damage using comet assay in patients successfully resuscitated from OHCA. Whether DNA damage measured using comet assay may be a prognostic marker remains unknown, although our data may encourage some suggestions. *(Anatol J Cardiol 2017; 18: 31-8)*

Keywords: cardiac arrest, out-of-hospital, DNA damage, comet assay, cardiopulmonary resuscitation, survivors

Introduction

There is a great emphasis on the need for early prognostication (at emergency room or before) of patients after out-of-hospital cardiac arrest (OHCA). Optimal early prognostic markers should be independent on both sufficient time for neurological recovery and major clinical status confounders (sedation, neuromuscular blockade, and metabolic derangements) (1). Early prognostic markers may be reliable only when a standardized, evidence-based post-cardiopulmonary resuscitation (CPR) treatment care is assumed. Prognostication is now based on a multimodal algorithm applied at ≥48 and ≥120 h after OHCA (1). However, the role of DNA integrity in prognostication of patients after OHCA remains unknown (2).

We aimed to investigate whether OHCA may induce severe DNA damage [single-strand breaks (SSBs) and double-strand breaks (DSBs)] measured using comet assay in successfully resuscitated humans and evaluate an OHCA short-term prognosis (30-day survival) using these DNA damage markers.

Methods

Design

This was a prospective, monocentric, controlled, blinded study (1/2013–1/2014). Inclusion and exclusion criteria have been previously reported (2). The patients enrolled in the study were all consecutive adults (age ≥18 years) who were successfully

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resuscitated [return of spontaneous circulation within 30 min, survival for ≥60 min following arrival at the emergency department (ED)] by professionals from non-traumatic OHCA of either cardiac or non-cardiac etiology (Table 1) and who met none of the exclusion criteria [active malignancy, the terminal phase of a chronic illness, toxic or suicidal causes (including drowning cases), chemotherapy or radiotherapy within the last year, and X-ray investigation within the last month or before the blood for DNA analysis was sampled). Healthy controls (all 10 consecutive volunteers) were also enrolled. Data were collected according to the Utstein style (3).

Ethics

The study complied with the principles of the 1975 Declaration of Helsinki, and the local Ethics Committee approved the study protocol. Patients' written informed consents were resolved with the aid of the law courts. Controls gave their written informed consents.

Patient group

A total of 41 patients [28 men; aged 63 (34–88), 63±14 years] together with 10 healthy controls [5 men; aged 55 (20–75), 53±17 years] were tested at admission for DNA damage (DSBs and SSBs) in peripheral lymphocytes using comet assay (Table 1). The majority of OHCA [78% (32/41)] was of cardiac etiology (Fig. 1, Table 1).

DNA analysis–comet assay

Peripheral blood samples for comet analysis were collected during the first 15 min (3 mL) after patients were transported to ED and always prior to X-ray examination or the commencement of therapeutic hypothermia. Heparinized venous blood was immediately processed for comet assay starting with lymphocyte isolation [using Histopaque 1077 (3 mL; Sigma-Aldrich, St. Luis, USA); centrifugation (at 400 G for 30 min at 20°C)]. Lymphocytes (the white ring on the surface of red cells) were washed [using phosphate buffered saline (PBS), 5 mL; three times], counted, and diluted to a concentration of 105 cells/mL.

DNA damage was measured using both alkaline and neutral versions of the comet assay (4). The alkaline version suitable for SSB detection has been described previously (5, 6). The neutral version suitable for DSB detection was a slight modification of that described in the papers of Olive et al. (7).

Briefly, cells embedded in 1% agarose (Sigma-Aldrich, St. Luis, USA) on microscope slides were lysed overnight at 4°C [1% Triton X-100 (Merck, Dermstadt, Germany), 2 500 mmol/L NaCl (Penta, Praha, Czech Republic, pH 10.0), 100 mmol/L EDTA (Penta, Praha, Czech Republic), and 10 mmol/L Tris (Penta, Praha, Czech Republic)]. Electrophoresis in the alkaline buffer (300 mmol/L NaOH; 1 mmol/L EDTA) was performed at 40 V, 300 mA for 30 min at 4°C after a 40-min period of unwinding. Electrophoresis in the neutral borate buffer (90 mmol/L Tris, 90 mmol/L boric acid, and 2 mmol/L EDTA; pH 8) was performed at 29 V, 6 mA for 40 min at 4°C

Table 1. Group characteristics of cardiopulmonary resuscitated individuals for out-of-hospital cardiac arrest (n=41)

Pre-hospital characteristics	**no. (%)**
Long-term medication before OHCA	
Diuretics	12 (29)
Beta-blockers	12 (29)
ACE inhibitors	19 (46)
Cigarette smoking	14 (34)
Initial cardiac rhythm	
Ventricular fibrillation	24 (59)
Asystole	10 (24)
Third-degree atrioventricular block	1 (2)
Pulseless electrical activity	6 (15)
Location	
Home	22 (54)
Public place (out of home)	19 (46)
Early electrical defibrillation	23 (56)
Arrest witnessed	34 (83)
Bystander CPR	28 (68)
Arrival time, min, call–ambulance arrival	
≤5 min	14 (34)
>5 min	27 (66)
Length of CPR by health care professionals	
0–15 min	17 (42)
16–30 min	24 (59)
Hospital characteristics	**no. (%)**
Cardiac OHCA etiology	
IHD without acute myocardial infarction	12 (29)
IHD acute myocardial infarction	11 (27)
Dilated cardiomyopathy	3 (7)
Idiopathic arrhythmia	3 (7)
Pulmonary embolism	2 (5)
Aortic dissection	1 (2)
Non-cardiac OHCA etiology	
Pneumonia	5 (12)
Stroke	3 (7)
Anaphylactic shock	1 (2)
Glasgow coma scale at admission	
3	37 (90)
4–5	2 (5)
≥6	2 (5)
STEMI	10 (24)
Primary PCI	9 (22)
Cardiogenic shock	15 (37)
Postanoxic encephalopathy	25 (61)
Left ventricular ejection fraction ≤ 35% (ECHO)	10 (24)
Emergent coronarography	20 (49)

ACE inhibitors - angiotensin-converting enzyme inhibitors; CPR - cardiopulmonary resuscitation; ECHO, echocardiography; IHD, ischemic heart disease; OHCA, out-of-hospital cardiac arrest; PCI, percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction

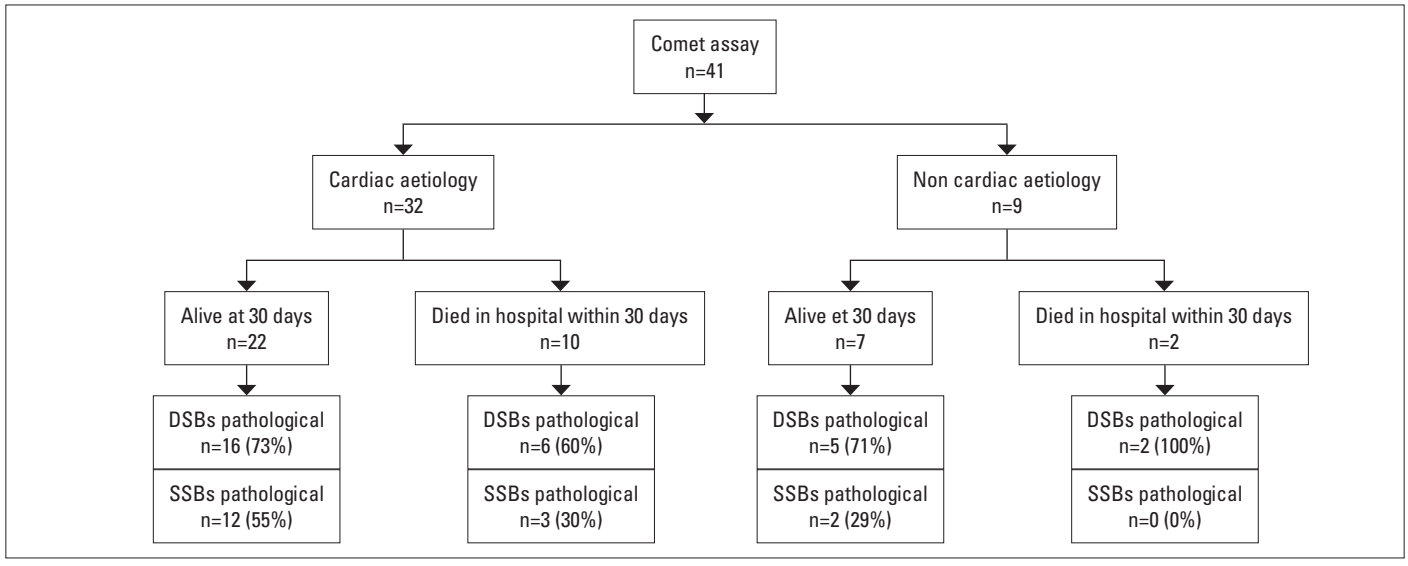

Figure 1. Frequency of short-term survivors (alive at 30 days) and pathological level of DNA breaks (double-strand breaks, DSBs; single-strand breaks, SSBs; both measured at admission using comet assay) after out-of-hospital cardiac arrest. Pathological DNA breaks were calculated from healthy controls (as more than "mean+2 SD": for DSBs >5.22% in tail and for SSBs >0.08% in tail). % in tail-percentage of DNA in comet tail

after washing out the borate buffer (three times). After neutralization (three times for 5 min; 400 mmol/L Tris–HCl, pH 7.5; once in distilled water), drying overnight on filter paper, and staining (ethidium bromide, 10 µL; 2.5 mmol/L; Sigma-Aldrich, St. Luis, USA), both comet versions were analyzed using fluorescence microscopy (excitation filter of 450–490 nm; suppression filter LP, 520 nm; 200× magnification). One hundred randomly selected lymphocytes were examined using the comet module of Lucia G image analysis (Laboratory Imaging, Prague, Czech Republic). Fifty cells per slide and two slides per patient were analyzed. The fluorescence intensity of the comet tail (DNA breaks) relative to the head (intact DNA) reflects the number of DNA breaks, expressed as the "percentage of DNA in comet tail" (% in tail). For each individual patient, the median from all values of "% in tail" were used.

Upper levels of normal ranges of DNA breaks (DSBs or SSBs) were calculated from parameters of healthy controls (mean+2 SD) and were 5.22% in tail and 0.08% in tail. Higher values were considered to be pathological.

Short-term prognosis

Good short-term prognosis was defined as 30-day survival. Poor short-term prognosis was defined as mortality during the first 30-day interval. The 30-day survival criterion following OHCA is a better indicator than that of hospital discharge, which was used in Utstein style (3).

Statistics

Categorical data are presented by absolute and relative counts and quantitative data by median (range) or mean±SD. Two-sample t-test or nonparametric Mann–Whitney U test, nonparametric Kruskal–Wallis analysis of variance with post-hoc Dunn's test with Bonferroni's modification, receiver operator characteristic (ROC) curves with the area under the curve (AUC), and 95% confidence interval (CI) were used. Univariate and multiple logistic regression analyses were also performed (however, multivariate analysis for independent predictors of 30-day survival failed). Level of significance was α=0.05. Statistical software NCSS 10 (NCSS LLC, Kaysville, Utah, USA, ncss.com/software/ncss) was used.

Results

Among 41 patients (Table 1, Fig. 1), there were 71% (29/41) survivors at day 30. The proportion of patients discharged alive was equal (71%). The length of hospitalization was 12 (1; 107); 18±20 days.

Double-strand breaks

The frequency of DSBs at pathological level has been shown in Figure 1. DSBs values and differences in these values between subgroups are presented in Figure 2a. Factors influencing DSBs were ventricular fibrillation and early electrical defibrillation, which lowered the number of DSBs and asystole (which increased the number of DSBs) (Table 2). The optimal cut-off value for prediction of short-term survival based on DSBs was 15.1% in tail (Fig. 3a).

Single-strand breaks

The pathological level for frequency of SSBs has been shown in Figure 1. SSBs values and differences in these values between subgroups are presented in Figure 2b. No factors influencing SSBs were found (Table 2). The optimal cut-off value for prediction of short-term survival based on SSBs was 0.15% in tail (Fig. 3b).

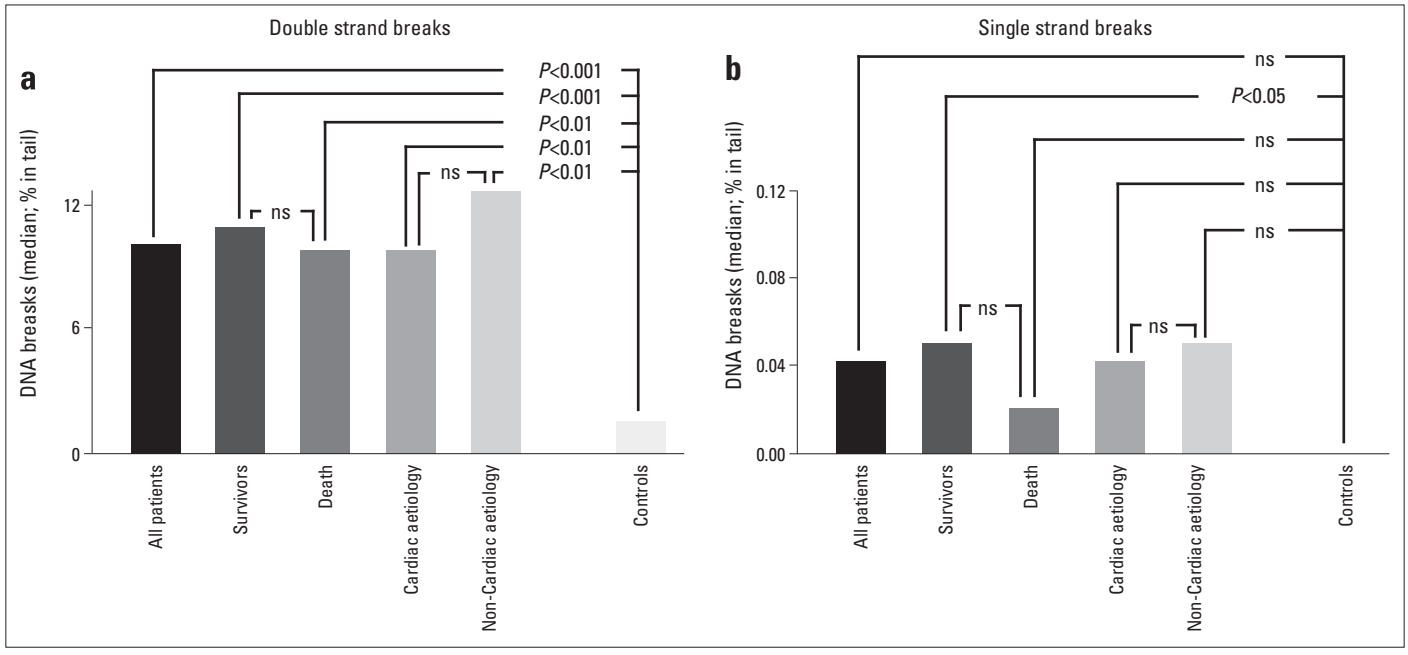

Figure 2. Differences in DNA damage (a. double-strand breaks, DSBs; b. single-strand breaks, SSBs; both measured at admission using comet assay) after out-of-hospital cardiac arrest and in healthy controls. In the order from "all patients" to "controls" DSBs expressed as median (range) mean±SD were: 10.1 (0.2–29.1) 11.0±7.6; 10.9 (0.2–29.1) 11.2±7.9; 9.9 (1.8–4.5) 10.6±7.4; 9.86 (0.15–29.1) 10.4±7.4; 12.7 (0.6–24.3) 13.4±8.5; and 1.33 (0.32–5.59) 1.96±1.63% in tail; SSBs were: 0.04 (0–11.4) 0.79±2.41; 0.05 (0–11.4) 1.81±3.69; 0.03 (0–0.36) 0.06±0.10; 0.05 (0–11.4) 0.98±2.75; 0.04 (0–1.03) 0.17±0.34; and 0 (0.00–0.09) 0.02±0.03% in tail. % in tail: percentage of DNA in comet tail

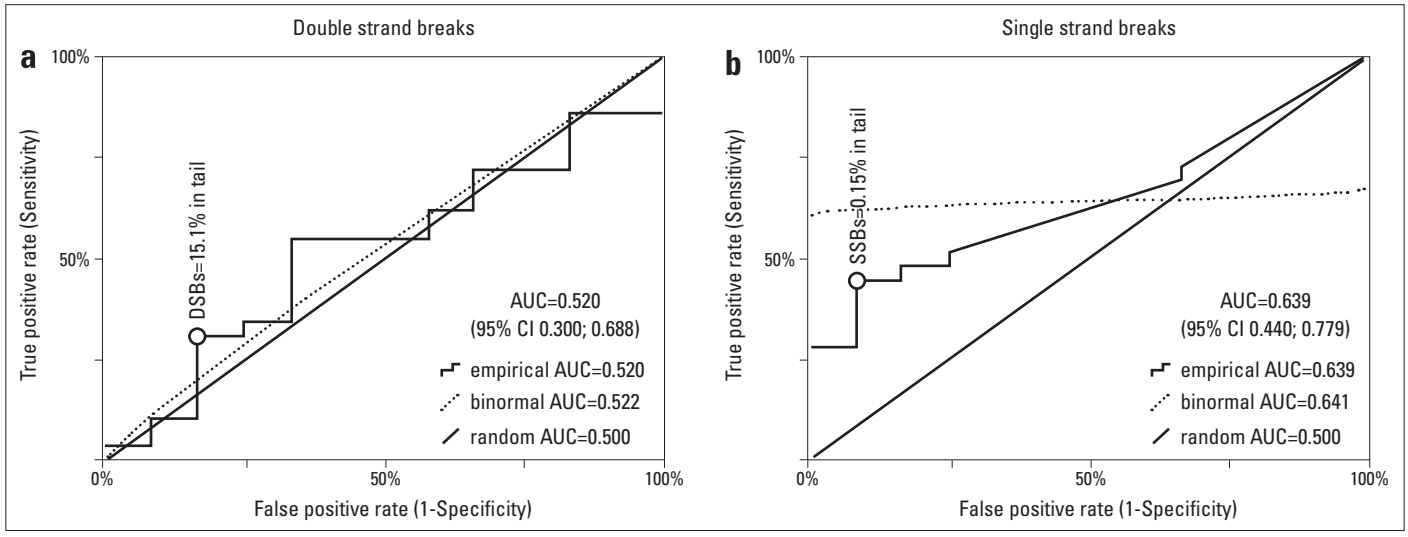

Figure 3. Receiver operating characteristics curve for DNA damage (a. double-strand breaks, DSBs; b. single-strand breaks, SSBs; both measured at admission using comet assay) to predict 30-day survival after out-of-hospital cardiac arrest. ○ The optimal cut-off value: for DSBs=15.1% in tail (according to the highest likelihood ratio of 1.862, with specificity of 83% and sensitivity of 31%) and for SSBs=0.15% in tail (according to the highest likelihood ratio of 5.379, with specificity of 92% and sensitivity of 45%)

AUC - area under curve; CI - confidence interval; DSBs - double-strand breaks; % in tail, percentage of DNA in comet tail; SSBs - single-strand breaks

Outcomes

Four factors including SSBs, ventricular fibrillation, length of CPR ≤15 min by health care professionals, and cardiogenic shock were found to be predictive of 30-day survival; no predictive value was found for other factors including DSBs (Table 3).

In contrast to DSBs (Fig. 3a; AUC=0.520), SSBs have a prognostic value (Fig. 3b; AUC=0.639).

Discussion

In the present study, the priority was to test the genotoxic effect of OHCA on intact and easily-available cells at admission and to evaluate the short-term prognostic role of induced genotoxicity (DNA damage: SSBs and DSBs) using comet assay. According to the findings of the present study, DNA damage was present in patients successfully resuscitated from OHCA.

Table 2. Impact of factors on DNA damage (double- and single-strand breaks; measured at admission using comet assay) after out-of-hospital cardiac arrest

Factors (yes versus no)	DSBs		DSBs		*P*	SSBs		SSBs		*P*
	Median	95% CI	Median	95% CI		Median	95% CI	Median	95% CI	
Men	10.8	5.3–14.2	8.0	2.0–15.0	0.39	0.04	0–0.15	0.05	0–0.37	0.89
Age ≥70 years	10.1	7.5–15.1	10.2	4.1–14.2	0.55	0.02	0–0.37	0.045	0.02–0.16	0.55
Diuretics	12.7	10.1–20.0	9.74	3.0–11.4	0.09	0.085	0.01–0.36	0.04	0–0.16	0.80
Beta-blockers	13.9	4.1–18.4	9.8	5.0–11.4	0.39	0.08	0–0.19	0.04	0–0.16	0.45
ACE inhibitors	11.3	4.4–16.3	9.8	3.0–14.2	0.32	0.02	0–0.15	0.045	0–0.19	0.68
Cigarette smoking	10.8	3.0–14.2	9.74	4.4–15.0	0.97	0.045	0–0.53	0.04	0–0.15	0.87
Ventricular fibrillation	9.7	4.1–11.4	12.7	8.0–22.6	**0.04**	0.045	0–0.19	0.04	0.01–0.14	0.82
Asystole	12.8	4.4–24.5	9.8	5.0–12.7	0.05	0.05	0–0.36	0.04	0–0.16	1.00
Location of arrest, home	7.8	2.2–10.9	14.2	9.7–16.3	0.07	0.05	0–0.36	0.04	0–0.11	0.52
Early electrical defibrillation	9.7	4.1–11.4	12.7	8.0–22.6	**0.04**	0.04	0–0.30	0.045	0.01–0.15	0.88
Arrest witnessed	10.4	6.7–12.7	10.1	0.2–24.5	0.98	0.05	0.02–0.16	0	0–0.36	0.42
Bystander CPR	10.0	4.1–12.7	10.7	4.4–22.7	0.74	0.04	0–0.15	0.06	0.01–0.32	0.69
Arrival time, ≤5 min	12.8	2.0–16.3	10.0	6.7–12.7	1.00	0.03	0–0.19	0.05	0–0.16	0.75
Length of CPR by health care professionals, ≤15 min	11.4	4.1–15.0	9.9	5.0–15.0	0.81	0.15	0.01–0.53	0.04	0–0.09	0.22
Hypokalemia at admission	13.1	8.0–18.4	9.7	4.1–12.7	0.26	0.075	0–0.19	0.04	0–0.30	0.75
STEMI	8.4	1.8–24.5	10.7	7.5–15.0	0.78	0.02	0–0.83	0.05	0.02–0.16	0.64
Cardiogenic shock	9.7	5.0–20.0	11.1	3.0–14.2	0.56	0.09	0.02–0.37	0.04	0–0.11	0.24
Postanoxic encephalopathy	10.0	5.3–15.0	10.8	2.2–15.0	0.64	0.04	0–0.14	0.085	0.01–0.30	0.32
Left ventricular EF ≤35%	10.0	5.0–18.4	10.9	4.4–15.0	0.94	0.055	0–0.19	0.04	0–0.30	0.73
Emergent coronarography	8.9	5.0–11.4	12.7	4.1–17.4	0.28	0.125	0.02–0.37	0.02	0–0.09	0.21
Cardiac etiology of OHCA	9.9	5.0–12.7	12.7	2.8–22.7	0.31	0.05	0.02–0.16	0.04	0–0.32	0.69
Survivors	10.9	5.3–15.0	9.9	4.4–15.0	0.82	0.06	0.10–0.30	0.03	0–0.09	0.16

ACE inhibitors - angiotensin-converting enzyme inhibitors; CI - confidence interval; CPR - cardiopulmonary resuscitation; DSBs - double-strand breaks (% of DNA in comet tail); EF - ejection fraction; OHCA - out-of-hospital cardiac arrest; *P* value of significance <0.05; SSBs - single-strand breaks (% of DNA in comet tail); STEMI - ST-segment elevation myocardial infarction; Two-sample t-test or nonparametric Mann–Whitney U test

Whether DNA damage is a prognostic marker remains unknown.

There are huge advances in acute care medicine, nevertheless the prognosis of patients after OHCA remains poor (8–10). The management of resuscitated patients is a very pressing issue, not only medically but also economically and ethically. Hence, it remains a clinical challenge to find an early predictor of prognosis after OHCA to facilitate reliable patient triage.

Despite the existence of several papers on DNA damage in cardiac disease (11–20), currently, very few papers have been published on genomic markers during and after OHCA (2, 21–24). White et al. (21) tested DNA from the cerebral cortex of dogs during their reperfusion following resuscitation for cardiac arrest, but no significant damage was found. However, a crucial limitation is that any study of DNA integrity in the cerebral cortex is inapplicable for clinical practice. In humans, the prognostic value of plasma cell-free DNA has recently been studied in patients after OHCA (22–24). Despite the small cohorts (81, 67, and 42 cases), the plasma cell-free DNA level detected using real-time polymerase chain reaction assay has been presented as a promising independent predictor of 24-h in-hospital mortality after OHCA (22–24). Unfortunately, the recommendation of the abovementioned studies is to collect samples for cell-free DNA level detection at 24 h after admission. We suggest that this is quite late for an early patient triage. Admittedly, it is compensated for by a slightly higher predictive value for 24-h hospital mortality (AUC=0.762) when comparing predictions based on samples provided at admission (AUC=0.636) (23). An additional limitation for clinical practice is that the major source of cell-free DNA circulating in the bloodstream are irreversibly destroyed cells, which are unable to reverse this unfavorable state.

In contrast to these works, our results clearly demonstrated OHCA genotoxicity (DNA damage) in intact cells, which is detectable prior to the destruction of the cells, and thus preserve the prospect for reversibility in case of appropriate therapeutic interventions. In a previous study, we detected DNA damage

Table 3. Analysis of risk factors for survival (univariate logistic regression)

Factors (yes versus no)	OR	R^2	95% CI	*P*	%
DSBs>optimal cut-off	1.90	0.075	0.34;10.7	0.447	44
SSBs>optimal cut-off	7.76	0.440	0.88; 68.4	**0.025**	56
Men	3.14	0.273	0.76; 13.0	0.112	68
Age ≥70 years	0.32	0.273	0.08; 1.31	0.112	68
Diuretics	0.76	0.018	0.18; 3.25	0.715	61
Beta-blockers	1.35	0.021	0.29; 6.20	0.697	44
ACE inhibitors	1.31	0.020	0.34; 5.09	0.699	51
Cigarette smoking	1.05	0.001	0.25; 4.37	0.944	44
Ventricular fibrillation	4.44	0.411	1.06; 18.7	**0.035**	68
Asystole	0.52	0.091	0.12; 2.34	0.400	66
Location of arrest, home	1.23	0.012	0.32; 4.74	0.763	54
Early electrical defibrillation	3.8	0.354	0.92; 15.8	0.058	66
Arrest witnessed	4.33	0.303	0.80; 23.6	0.088	73
Bystander CPR	3.14	0.273	0.76; 13.0	0.112	68
Arrival time, ≤5 min	0.63	0.055	0.16; 2.53	0.517	61
Length of CPR by health care professionals, ≤15 min	13.54	0.614	1.54; 119	**0.003**	66
Hypokalemia at admission	4.95	0.290	0.55; 44.4	0.098	49
X-ray	1.23	0.012	0.32; 4.74	0.763	54
STEMI	0.52	0.091	0.12; 2.34	0.400	66
Cardiogenic shock	0.042	0.798	0.007; 0.25	**0.001**	83
Postanoxic encephalopathy	Calculation has failed				
Left ventricular EF ≤35%	0.52	0.091	0.12; 2.34	0.400	66
Emergent coronarography	0.93	0.001	0.24; 3.58	0.920	51

ACE inhibitors - angiotensin-converting enzyme inhibitors; CI - confidence interval; CPR - cardiopulmonary resuscitation; DSBs - double-strand breaks (comet assay); DSBs>optimal cut-off, value of DSBs higher than receiver operating curve optimal cut-off value, which is 15.1% of deoxyribonucleic acid in tail; EF - ejection fraction; OR - odds ratio; %, percent of the true classification; R^2 - the proportion of variation in the dependent variable accounted for by the independent variables; SSBs - single-strand breaks (comet assay); SSBs>optimal cut-off, value of SSBs higher than receiver operating curve optimal cut-off value, which is 0.15% of deoxyribonucleic acid in comet tail; STEMI - ST-segment elevation myocardial infarction; Results are employed for survival (thus OR>0 indicates a higher chance for survival; OR<0 indicates a lower chance for survival)

(using γH2AX method) at admission in a majority of successfully resuscitated patients from OHCA (83%) (2). Similar results were found in this work (using comet assay). When comparing controls, for DSBs, significant differences were found for both patient groups, whereas for SSBs, the impact of OHCA on DNA damage was less apparent (a partial explanation could be the lower range of quantified DNA damage).

We did not find apparent differences in DNA damage between cardiac and non-cardiac etiology of OHCA or between surviving and non-surviving patients using either γH2AX (2) or the comet assay method (this study). In contrast to γH2AX, which is an indirect marker of DNA damage (γH2AX positivity reflects the intensive DNA reparation) (2), the comet assay directly displays DNA damage.

When comparing cell-free DNA, the authors assumed based of pathophysiology that the optimal time at which analysis of blood samples should be performed for DNA damage should be shortly (minutes or hours) after OHCA. Unfortunately, serial measurements of DNA damage after OHCA have not been provided in our work. It was shown by others (25, 26) that maximum DNA changes occur 60–180 min after exposure to some factors (namely isoflurane anesthesia); therefore, it might be beneficial to sample the blood in OHCA patients later than at admission (our study) to get better predictive results. Also, Corbucci et al. (27) detected still-rising DNA damage during 100 min of controlled ischemia-reperfusion exposure. Unfortunately, the DNA damage was measured in human cardiac myocytes, which are unavailable material, similarly to a study by Bartunek et al. (27, 28). On the other hand, using this protocol with samples collected 60–180 minutes after OHCA may be rendered invalid in practice because of exposure to other diagnostic and therapeutic factors, which may induce DNA damage (e.g., X-ray; the routine practice usually includes emergent angiography or computer tomography). However, at admission, both DNA breaks and cell-free DNA give comparable predictive values of hospital mortality (AUC for SSBs with comet assay: 0.639; DSBs with comet assay: 0.520; γH2AX: 0.602; cell-free DNA: 0.636) (2, 23). For appropriate interpretation, one should be aware that Gornik et al. (23) calculated the predictive power from 24-h mortality, not from in-hospital mortality, despite there being an apparent difference between the two (24-h mortality, 37% vs. in-hospital mortality, 72%; the length of hospitalization in Gornik´s study was not specified) (23). In our study, the predictive value was calculated from 30-day mortality (32%).

Among the Utstein style parameters (3), the differences in DSB results (comet assay) were applied to three factors (ventricular fibrillation as the initial rhythm, early electrical defibrillation resulting in lower DSBs, and asystole as the initial rhythm increasing the number of DSBs), while in the γH2AX method, the difference was applied to the initial rhythm (2). In contrast to DSBs, no differences were found at this point in SSBs (comet assay).

Analysis of the association between survival at day 30 association and the Utstein style factors was in our small study the only marginal matter. Nevertheless, our results concur with the literature: the direct association between 30-day survival and ventricular fibrillation or length of CPR by health care professionals ≤ 15 min and the indirect association between 30-day survival and cardiogenic shock. However, this study is the first to describe the direct association between survival at day 30 and SSBs.

The prognostic role of DNA damage in patients successfully resuscitated from OHCA remains unknown. Despite this, our study suggests a useful prognostic potential for DNA damage analyzed using either comet assay or the γH2AX method (AUC, 0.520–0.639) (2). These results should be verified in a future study including serial measurements and comparison with cell-free DNA results (22–24).

Based on our results, we hypothesize that DNA damage (assessed using comet assay or the γH2AX method) is a more sensitive marker for post-resuscitation outcomes when compared with the cell-free DNA level.

Study limitations

The present study has several methodological limitations. The primary limitation is that it was a single-center study with a small number of participants, which is a consequence of the prospective and pilot design of the study. Another limitation is that the control group was not matched to the cohort. Despite that, the authors judge the control group to be adequate because numerous literature data confirmed that under normal conditions, lymphocytes show a low background level of SSBs or DSBs (29). The main question, which our results have opened up, is regarding the best timing for collection of DNA damage samples.

Conclusion

In conclusion, our study demonstrated significant DNA damage, especially for DSBs, measured using comet assay in patients successfully resuscitated from OHCA compared with that in controls with no dependency on the cardiac arrest etiology.

The prognostic value of DNA damage remains unknown, although our results suggest a potential usefulness. Future research should include serial measurements of DNA damage at admission and later to test the influence of DNA damage dynamics on post-arrest patient outcomes.

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