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Severe deoxyribonucleic acid damage after out-of-hospital cardiac arrest in successfully resuscitated humans

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Out-of-hospital cardiac arrest (OHCA) is a highly stressful event with a transient common ischaemic — reperfusion injury in successfully resuscitated victims. Survival rate of patients resuscitated from OHCA remains too low (10%) [1]. It has been shown that stress induces the most severe form of deoxyribonucleic acid (DNA) damage — double strand breaks (DSBs) [2–5]. The reliable marker of DNA DSB damage, respectively its reparation is the phosphorylated histone H2AX (γH2AX), which reaches maximum levels in peripheral blood lymphocytes between 10 and 60 min following cell exposure [3–8]. The effect of OHCA on DNA integrity has not been described. Thus the aim of the authors was to describe in patients successfully resuscitated from OHCA the occurrence of DNA DSB damage and evaluate γH2AX short-term prognostic (30-day survival) role.

A prospective, monocentric controlled and blinded study was performed (January 2013–January 2014). Enrolled in the study were all consecutive patients who were successfully resuscitated by professionals from non-traumatic OHCA (age ≥ 18 years, return of spontaneous circulation within 30 min, survival for ≥60 min following arrival at the emergency department/ED/). The exclusion criteria were: toxic or suicidal causes, including drowning cases, the terminal phase of a chronic illness, radiotherapy or chemotherapy within the last year, active malignancy, and X-ray investigation within the last month or before the blood for DNA analysis was sampled.

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.

A total of 41 patients (30 men; aged 33–88 years; median 67; average 64 ± 14) as well as 13 healthy controls (10 men; aged 25–56 years; median 40; average; 40 ± 10) were analysed using γH2AX in peripheral lymphocytes (Table 1).

Peripheral blood samples for γH2AX analysis were obtained during the first 15 min (10 ml) after patients were transported to the ED. The

Table 1
Characteristics of study population (n = 41).

Initial cardiac rhythm — no. (%)	
Ventricular fibrillation	26 (63)
Asystole	10 (24)
Third-degree atrioventricular block	1 (2)
Pulseless electrical activity	4 (10)
Location — no. (%)	
Home	20 (49)
Public place (out of home)	21 (51)
Arrest witnessed — no. (%)	36 (88)
Bystander CPR — no. (%)	30 (73)
Arrival time (min) (call — ambulance arrival) — no. (%)	
≤5 min	17 (42)
>5 min	24 (59)
Glasgow coma scale at admission — no. (%)	
3	37 (90)
4–5	2 (5)
≥6	2 (5)
Cardiogenic shock — no. (%)	16 (39)
Postanoxic encephalopathy — no. (%)	26 (63)
Main diagnosis — no. (%)	
Cardiac aetiology	
IHD without acute myocardial infarction	13 (32)
IHD acute myocardial infarction	9 (22)
Dilated cardiomyopathy	3 (7)
Others	6 (15)
Non-cardiac aetiology	
Pneumonia	7 (17)
Stroke	2 (5)
Anaphylactic shock	1 (2)

CPR: cardio-pulmonary resuscitation; IHD: ischaemic heart disease.

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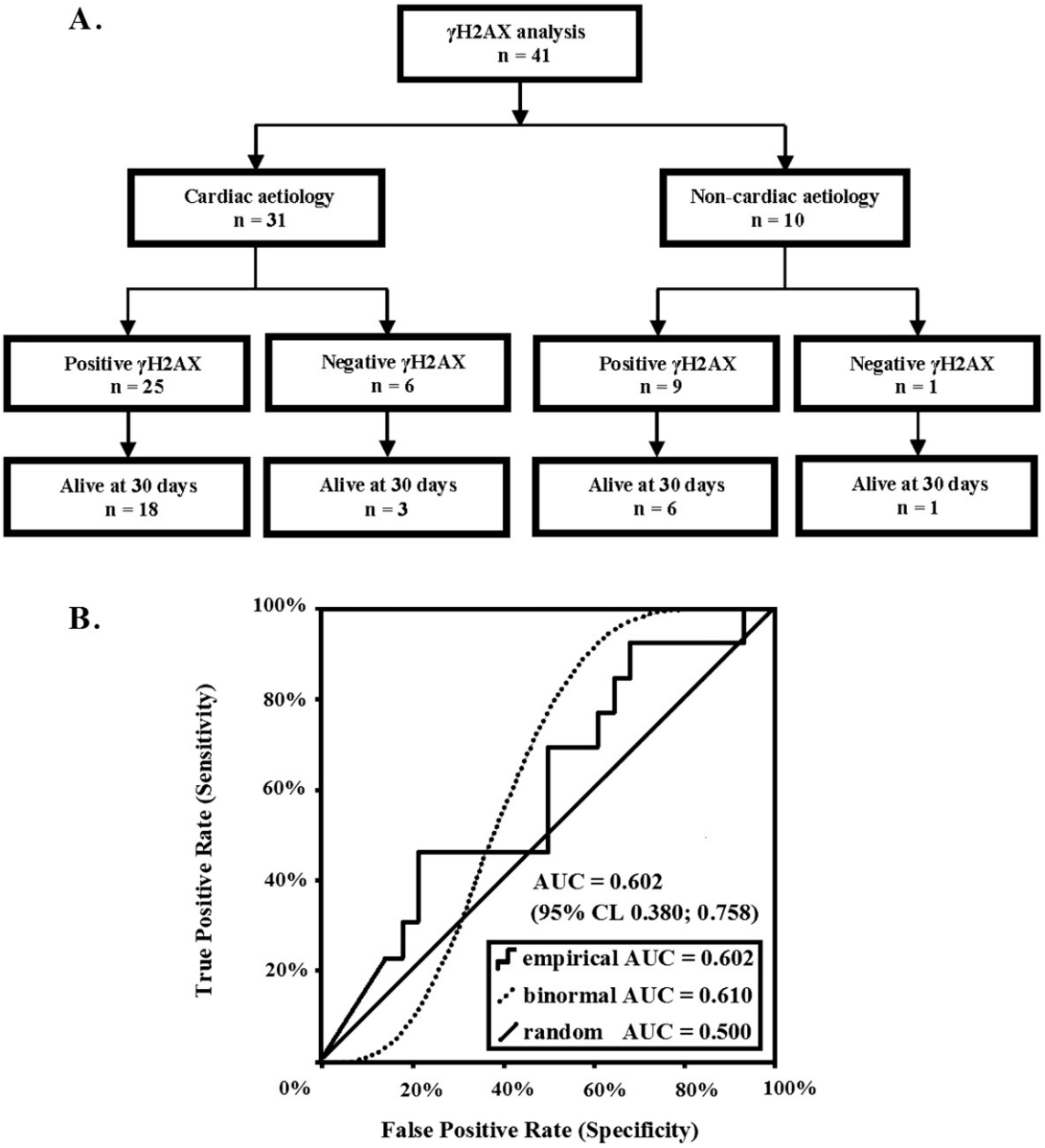

Fig. 1. Patient group after out-of-hospital cardiac arrest: γH2AX results and 30-day outcomes (A), receiver operating characteristics curve for γH2AX positivity (intensive reparation of the most severe deoxyribonucleic acid damage) at admission to predict 30-day survival (B). γH2AX: double strand break (DSB) indicator. Positive γH2AX: median DSBs > 0. Negative γH2AX: median DSBs = 0. AUC: area under curve.

method in details has been described previously [2,9]. Briefly, lymphocytes were isolated from heparinised peripheral blood.

For immunocytochemical detection of γH2AX the lymphocytes were washed using phosphate buffer saline (PBS) and fixed with fresh 4% paraformaldehyde. Cells were blocked in solution with 7% inactivated foetal calf serum and 2% bovine serum albumin and immunostained with appropriate primary antibodies. After pre-incubation with 5.5% donkey serum the secondary antibody was applied to each slide. The nuclei were counterstained with 4′,6-diamidino-2-phenylindole solution, incubated, washed and fixed. Images were obtained by Nikon Eclipse fluorescence microscope. Foci of γH2AX in the nuclei of lymphocytes were detected using automated image processing and measured as the integrated optical density (IOD), which is the automated image analysis algorithm incorporated into the specialized software ImagePro 4.11 (MediaCybernetics, USA).

The number of examined lymphocytes was ≥100 in each patient. Based on the blindly measured values of γH2AX IOD in lymphocytic nuclei the mean and median value per one nucleus was established for each patient. The test for γH2AX was considered to be "negative" when the calculated median of the γH2AX IOD was 0.0. In the healthy control group the median of the IOD γH2AX in each of them was 0.0.

Results are presented by absolute and relative counts, mean ± SD or median. Fisher's exact test two-proportions test (statistical software NCSS 9; $P < 0.05$ of statistical significance), receiver operator characteristic (ROC) curves with the area under the curve (AUC) and 95% confidence interval (CI) were used.

DNA results (positive/negative γH2AX) as well as 30-day survival are summarized in Fig. 1A. DNA damage was seen in a majority of patients independently on OHCA aetiology (all patients: 34/41 = 82.9%; cardiac aetiology: 25/31 = 80.7%; non-cardiac aetiology: 9/10 = 90.0%)

(Fig. 1A). Of patients with positive γH2AX (n = 34): 70.6% (24/34) were survivors at day 30, whereas in the subgroup with negative γH2AX (n = 7): 57.1% (4/7) patients survived day 30 (P = 0.659) (Fig. 1A). Of survivors at day 30 (n = 28): 85.7% (24/28) had positive γH2AX, whilst of patients who died in hospital (n = 13): 76.9% (10/13) had positive γH2AX (P = 0.659). The power of positive γH2AX to predict 30-day survival in patients admitted to hospital (AUC 0.602) is demonstrated in Fig. 1B. Among the Utstein style parameters [10] the differences in DNA results were only applied to the initial rhythm (positive γH2AX was more frequently in ventricular fibrillation than in asystole: 92.3% (24/26) vs. 60% (6/10); P = 0.039).

In conclusion, the authors demonstrated for the first time severe DNA damage in a majority (83%) of patients successfully resuscitated from OHCA. Furthermore, the authors bring a suggestion that intensive DNA DSB reparation (γH2AX positivity) reflects good short-term prognosis of resuscitated patients (survivors seem to be more frequent when γH2AX is positive: 71% vs. 57%; AUC 0.602). Our results need to be confirmed by additional research.

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Conflicts of interest

The authors report no relationships that could be construed as a conflict of interest.

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