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ON THE USE OF BIOCHEMICAL MARKERS IN DIAGNOSTICS OF SUDDEN CARDIAC DEATH

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Summary

Introduction: Medical examiners frequently examine victims of sudden death. Most often sudden deaths have a cardiovascular cause. To determine the diagnosis of sudden cardiac death based only on morphological findings may be often very difficult. Measurement of blood concentrations of cardiac troponin I (cTnI) and atrial natriuretic peptide (pro-ANP) is now in clinical use in adult patients with heart failure caused by myocardial damage.
Aim: The aim of the study was the estimation wheather cTnI and/or pro-ANP could be markers of sudden cardiac death.
Patients and methods: The study was carried out on 89 necroptic cases, of which 53 were concluded as cardiac-related sudden death, and 36 cases were used as a control group being other than cardiac death cases. Concentrations of markers were determined in blood taken from the left cardiac ventricle and from the right femoral vein. The dependence between the results of biochemical studies and death causes, results of histopathological examination of myocardium, time interval between the death and taking of samples, and resuscitation data was investigated.
Results: Concentrations of cTnI as determined in blood samples from the left ventricle were in most cases very high, largely exceeding the cut-off level, and so were concentrations of pro-ANP. The values of both parameters were significantly lower in peripheral blood. No statistically significant dependences were found between the levels of the studied markers and the cause of death, myocardial histopathological findings, time interval between the death and taking of samples, and resuscitation data.
Conclusion: Based on the results obtained, the study can be concluded that blood is not a suitable medium for determination of biochemical markers of cardial troponin I and atrial natriuretic peptide for post-mortem diagnostics of myocardial damage and for determining the diagnosis of sudden cardiac death in a manner similar to diagnostics of myocardium damage in living patients.

Key words: sudden cardiac death – morphology - biochemical markers – cardiac troponin I – atrial natriuretic peptide

Súhrn

Využitie biochemických markerov v diagnostike náhlej srdcovej smrti

Úvod: Vyšetrovanie prípadov náhlych úmrtí je často predmetom súdnolekárskej praxe. V mnohých prípadoch ide o úmrtia z kardiovaskulárnych príčin. Stanoviť diagnózu náhlej srdcovej smrti len na základe morfologických nálezov môže byť niekedy veľmi obtiažne až nemožné. V diagnostike poškodenia myokardu u živých pacientov sa bežne využívajú biochemické markery kardiálny troponín I (cTnI) a atriálny natriuretický peptid (pro-ANP). Cieľ: Cieľom práce bolo overiť využitie biochemických markerov cTnI a pro-ANP v diagnostike náhlej srdcovej smrti.
Pacienti a metódy: Predmetom štúdie bolo 89 nekroptických prípadov. V 53 prípadoch išlo o náhlu srdcovú smrť, 36 prípadov s inou ako kardiálnou príčinou smrti slúžilo ako kontrolný súbor. Koncentrácie markerov boli stanovené v krvi z ľavej komory srdca a pravej femorálnej vény. Bola zisťovaná závislosť medzi výsledkami biochemického vyšetrenia a príčinami smrti, morfologickými nálezmi v myokarde, časovým intervalom medzi smrťou a odberom vzoriek biologických materiálov a údajmi o resuscitácii.
Výsledky: Koncentrácie cTnI zistené vo vzorkách krvi z ľavej komory srdca v prevažnej väčšine prípadov mnohonásobne prevyšovali cut-off hodnoty, podobne ako koncentrácie pro-ANP. Hodnoty obidvoch parametrov boli významne nižšie v periférnej krvi. Neboli zistené signifikantné vzťahy medzi hodnotami sledovaných markerov a príčinou smrti, histopatologickými nálezmi v myokarde, časovým intervalom medzi smrťou a odberom vzoriek a údajmi o resucitácii.
Záver: Na základe získaných výsledkov je možné konštatovať, že krv nie je vhodný materiál na stanovenie biochemických markerov kardiálneho troponínu I a atriálneho natriuretického peptidu pre stanovenie diagnózy náhlej srdcovej smrti podobne ako pri zisťovaní poškodenia myokardu u klinických pacientov.

Kľúčové slová: náhla srdcová smrť – morfológia – biochemické markery – kardiálny troponín I – atriálny natriuretický peptid

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INTRODUCTION

Medical examiners frequently examine victims of sudden death. From a forensic point of view, sudden death is currently most often defined as rapid, unexpected and natural death occurring within 1 h of the onset of final symptoms (5). The time elapsing between the onset of final symptoms and death is controversial, settled at 24, 6 or 1 h according to different definitions. However, such determination is often useless

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because sudden death is unwitnessed in about 40% of cases (16), for example when it occurs during sleep. The time period between onset of symptoms and death is therefore frequently unknown. Most often sudden deaths have a cardiovascular cause when the time period is short (5), although extra cardiac causes may be encountered.

From a general perspective, morphological diagnostics of cardiac-related sudden death can be divided into three categories. In some cases, a firm diagnosis of cardiac-related death can be made based on conclusive gross and histopathological findings. In many other cases, we find evidence supportive, but not diagnostic, of cardiac death (e.g., atherosclerotic coronary artery disease, cardiomegaly, myocardial scarring). The third cohort comprises cases of sudden death with minimal to mild cardiac disease, no other significant pathology, and negative toxicology studies (3).

In cases where sudden death occurs at a very early stage of infarction, the myocardial lesions cannot be easily detected by traditional macroscopic examination or routine histopathological staining. Thus, examination of haematoxylin-eosin-coloured slides will not, with certainty, reveal ischaemic lesions until more than 6 h post-infarction (9, 10). In an attempt to improve the detection of early myocardial infarctions, several immunohistochemical techniques have been introduced and have contributed some improvement into the detection of early ischemic lesions (4, 7, 11). Nevertheless, a marker with both high sensitivity and high specificity has not been found.

Several biochemical markers, such as creatine kinase MB, myoglobin, cardiac troponins, natriuretic peptides and others are used for the diagnosis of myocardial injury in clinical practice (13).

In forensic medicine, there is a need for sensitive biochemical markers for the post-mortem diagnosis of cardiac-related sudden death.

Troponins (T, I, C) are structural proteins bound to tropomyosin of striated muscles. They take part in regulation of muscle contraction. The primary structure of myocardial troponin was found to differ from that of the skeletal muscle troponin, and the two species can be immunochemically differentiated. This provides a cardiospecific method of differentiating myocardial and skeletal muscle lesions. So far, the largest body of experience has been acquired with determinations of cardial troponins T (cTnT) and I (cTnI). They are markers of microscopic myocardial necroses (14). The release of troponins into circulation as a result of traumatic heart lesion has been repeatedly reported (1, 2, 6).

Atrial natriuretic peptides ANP, BNP, and CNP are pro-hormones produced by cardiomyocytes of cardiac atria. Together with their receptors A, B, and C play an important role in maintaining the homeostasis. They have vasodilating, diuretic and natriuretic actions and regulate the systemic resistance. These peptides have been proved to contribute to pathogenesis of many diseases, for example heart failure, hypertension, acute coronary events etc. They are markers of myocardial ischaemia and, in clinical medicine, their concentrations in adult patients with cardiac failure are monitored (12).

AIM

The aim of the study was to evaluate and compare the diagnostic efficacy of post-mortem cTnI and pro-ANP determinations in the serum of heart and peripheral blood, to compare these results with structural findings in myocardium, and to estimate whether these biochemical markers could be used for post-mortem diagnosis of cardiac-related sudden death.

PATIENTS

The study was carried out on 89 necroptic cases, of which 53 were concluded as cardiac-related sudden death, and 36 cases were used as a control group being other than cardiac death cases. The time interval from the death to taking of biological samples (blood and myocardium) was 6 to 105.5 hours. We also recorded data on resuscitation.

METHODS

Concentrations of markers were determined in blood taken from the left cardiac ventricle and from the right femoral vein. The samples were centrifuged, the supernatant was divided in 3 parts and stored at -20 °C. Using an immunochemical chemiluminiscence assay, we determined the concentration of cTnI using mouse monoclonal antibodies ACS-180 (Bayer, Germany) with threshold concentration (cut-off for healthy people – 0.15 ng/ml), and the concentration of pro-ANP using sheep polyclonal antibodies – LIAmat assay SERISTRA diagnostic kits (B.R.A.H.M.S. AG, Germany) with cut-off of 200 pmol/l. At the same time, samples of myocardium were taken; these were fixed in formalin, processed using a routine procedure by embedding in paraffin blocks and staining with haematoxylin-eosin and phosphotungstic acid according to Mallory. The samples were examined using light microscopy.

We investigated the dependence between the results of biochemical studies and death causes, results of histopathological examination of myocardium, time interval between the death and taking of samples, and resuscitation data.

STATISTICS

For observed marker levels we report basic statistics (mean, standard deviation, range, and median). We note that source data in this study were seriously censored. The reason is that the observed levels were frequently perceived as unreasonably large. Pairwise comparisons between groups were carried out using the Wilcoxon-Mann-Whitney U-test and the Kolmogorov-Smirnov two-sample test (17). As the results were identical, only the results of U-test are reported. To evaluate the diagnostic potential of the two markers, we report maximum absolute deviation (MAD) between the empirical distribution functions of the sudden cardiac death and control groups. This quantity is known as a test statistic in the Kolmogorov-Smirnov two-sample test. For the purposes of this paper, its important property is that it gives the probability of correct classification of a case (sudden cardiac death vs. other cause of death) based on observed level of a marker. A diagnostically usable marker would provide values of MAD close to 1 (meaning correct classification with probability close to 1). Due to censoring in the data, we constructed the empirical distribution functions used in the calculation of MAD as Kaplan-Meier estimates (8). The calculations were carried out in the R framework (15).

RESULTS

Concentrations of the two markers as determined in blood samples from all cases examined are shown in Table 1. Data are compared by cause of death (sudden cardiac death vs. other), and we report, along with basic descriptive statistics, results of the Wilcoxon-Mann-Whitney U-test and maximum

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Table 1. Observed levels of cTnI and pro-ANP in blood taken from the left ventricle and from v. femoralis for the sudden cardiac death cases (n = 53) and for the control group (n = 36). We report p-values for the Wilcoxon-Mann-Whitney comparisons between groups. MAD are probabilities of correct classification (sudden cardiac death vs. other cause of death) of a case based on the corresponding marker level

	Blood from the heart				Blood from v. femoralis			
	cTnI (pg/l)		pro-ANP (pmol/l)		cTnI (pg/l)		pro-ANP (pmol/l)	
	Sudden death	Control group	Sudden death	Control group	Sudden death	Control group	Sudden death	Control group
Range	15–11000	28–11000	708–3139	0–3139	0–11000	0–377	108–3139	4–1446
Mean	6143	4598	2840	2170	382	43	567	309
STD	4123	3979	528	1040	1507	89	585	330
Median	5500	4653	3139	2435	40	7	352	179
U-test p	0.11		0.005		0.004		0.003	
MAD	0.45		0.41		0.33		0.33	

absolute deviations between distribution functions. The concentrations of cTnI in the blood from the left ventricle were in most cases very high, and so were concentrations of pro-ANP. The values of both parameters were significantly lower in peripheral blood. However, even these were by at least an order of magnitude higher than the cut-off values mentioned above. An interesting finding was a low level of pro-ANP in the blood from v. femoralis in the subgroup (n = 12) with asphyxia as cause of death, as shown in Table 2.

The MAD values indicate that the observed levels of the two markers have poor predictive value as regards the cause of death. Due to the broad distributions of observed levels, a specific level of a marker bears practically little information about the cause of death. This holds irrespective of whether the differences between the sudden cardiac death group and control group are found significant.

No statistically significant dependences were found between the levels of the studied markers and the cause of death, time interval between the death and taking of samples, or resuscitation data. Similarly, no significant correlation was identified between the marker levels and the findings of histopathological examination of myocardium, which provided a broad range of findings, from negative through minor ischaemic alterations to developed infarctions – that is, necrosis.

Table 2. Observed levels of cTnI and pro-ANP in blood taken from v. femoralis for cases who died of suffocation (n = 12) or for other reason. We report p-values for the Wilcoxon-Mann-Whitney comparisons between groups. MAD are probabilities of correct classification (sudden cardiac death vs. other cause of death) of a case based on the corresponding marker level

	cTnI (pg/l)		pro-ANP (pmol/l)	
	Asphyxia	Other	Asphyxia	Other
Range	0–43	0–11000	23–983	4–3139
Mean	7.6	254	189	470
STD	11.8	1202	259	519
Median	3	17	109	259
U-test p	0.004		0.001	
MAD	0.46		0.48	

DISCUSSION AND CONCLUSIONS

We observed that, in all cases and irrespective of the cause of death, the levels of the two markers studied covered a wide range largely exceeding the cut-off level for the respective marker. The effect was less pronounced for pro-ANP than for cTnI.

High concentrations of the markers in blood samples from the left cardiac ventricle in both the sudden cardiac death and control group can be attributed to post-mortem alterations, such as haemolysis, which can take place shortly after death. The reason for positive findings and increased levels also in peripheral blood samples is largely unclear.

Though the levels of both markers were in most cases significantly higher in the sudden cardiac death group compared to the control group, the ranges of values were always largely overlapping, making diagnosis based on marker levels impossible, as confirmed by the MAD values.

Based on our observations, we conclude that blood is not a suitable medium for determination of biochemical markers of cardial troponin I and atrial natriuretic peptide for post-mortem diagnostics of myocardial damage and for determining the diagnosis of sudden cardiac death in a manner similar to diagnostics of myocardium damage in living patients. This overall conclusion pertains to both cTnI and pro-ANP.

The interesting finding of close-to-normal levels of pro-ANP in peripheral blood in cases who died of asphyxia is worth further study.

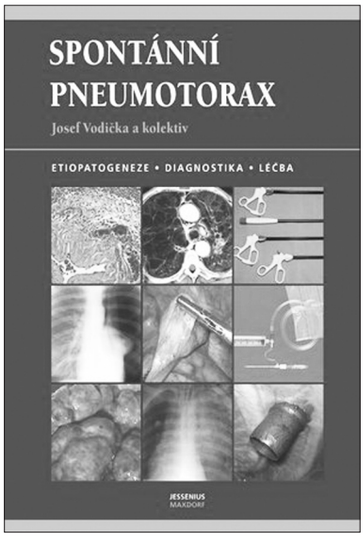
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SPONTÁNNÍ PNEUMOTORAX

Etiopatogeneze, diagnostika a léčba

Josef Vodička a kolektiv

Spontánní pneumotorax je definován jako patologické nahromadění vzduchu v pohrudniční dutině, ke kterému dochází bez zjevného zevního mechanického zásahu. Svojí povahou toto onemocnění zasahuje jak do oboru chirurgie, tak do oboru pneumologie. Diagnostika a především pak léčba spontánního pneumotoraxu prošly v uplynulých letech řadou zásadních změn. Cílem autorů je podat v této knize vyčerpávající a moderní výklad dané problematiky, neboť monografie podobného obsahu nebyla u nás dosud vydána.

V úvodní části je připomenuta embryologie, anatomie a fyziologie dýchací soustavy, v druhé je pak podrobně rozebrána problematika spontánního pneumotoraxu počínaje etiopatogenezí, přes diagnostiku, až po prognózu. Zásadní částí knihy je kapitola věnovaná terapii tohoto onemocnění, neméně důležitá je i kvalitní obrazová dokumentace. Svou koncepcí a náplní je jedinečným dílem, které v takové podobě poprvé poskytuje souhrnné a přitom nejmodernější poznatky o tomto onemocnění. Proto by tato publikace neměla chybět v knihovně žádného chirurga či pneumologa. Mnoho užitečných poznatků může nepochybně přinést i široké lékařské veřejnosti, včetně studentů medicíny.

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