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Cardiac and renal markers: reference population versus marathon runners

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Introduction

Regular exercise belongs to a healthy life-style and is widely accepted to prevent cardiovascular diseases. Nevertheless, cardiac markers, like the highly specific cardiac troponins (cTn), are known to be elevated after prolonged exercise up to even minor myocardial infarction concentrations (1, 2). Since the consequences are still unexplained, exercise-induced cTn release is widely discussed these days (3) and requires further investigation.

At the moment most cTn assays lack sufficient analytical sensitivity to detect cTn in the lower range (<0.01 µg/L) (4, 5), like in healthy individuals and after strenuous exercise. The National Academy of Clinical Biochemistry recommends that cTn assays should strive for a CV <10% at the 99th percentile of a reference population (6). Consequently, more sensitive cTn assays have recently been developed and have been evaluated in different clinical settings (7, 8).

Here, we investigated the analytical performance of two recently introduced cTn assays, the pre-commercial 'high-sensitive' hs-cTnT assay from Roche Diagnostics and the cTnI-Architect assay from Abbott Diagnostics. CTn concentrations were investigated in a reference population and in a cohort of marathon runners. Currently available cTn assays and NT-proBNP measurements were included for comparison. In addition, creatinine and cystatin C concentrations were incorporated to study if exercise-induced cTn release could be the effect of a reduced renal function.

Methods

The reference population consisted of 501 apparently healthy persons. To rule out individuals with cardiac syndromes, 21 subjects were excluded exceeding mean + 3 SD for CK-MB mass, NT-proBNP or cTn. The marathon study population consisted of 85 runners (70 males, 15 females). All signed the informed consent. This study was approved by the local ethical committee. Serum was collected <2 h prior to the run, <1 h post-run and for 23 subjects also the day after the run. Aliquots were stored at -80 ºC. Post-run concentrations were albumin corrected.

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CTnI was measured on the Immulite 2000 (Siemens Laboratory Diagnostics) and on the Architect i2000SR (Abbott Diagnostics). CTnT was measured on the Elecsys 2010 (Roche Diagnostics) using the 4th generation cTnT immunoassay and the pre-commercial hs-cTnT assay. In addition, NT-proBNP was measured on the Elecsys 2010, CK, albumin and creatinine on the Synchron LX 20 (Beckman Coulter) and cystatin C on the BN ProSpec (Siemens). Assay characteristics are summarized in table 1.

Data analysis was performed using SPSS, Version 13.0. The 97.5th and 99th percentile were calculated non-parametrically. Parameters with a Gaussian distribution (Kolmogorov-Smirnov normality test) were tested using the paired-samples *t*-test and the independent-samples *t*-test. The non-Gaussian distributions were log-transformed. In case normality was not obtained, the original data were tested using the Wilcoxon Signed-Rank test and the Mann-Whitney test. For statistical comparison, cTnT and cTnI-Immulite concentrations below the limits of detection (<LOD) were set equal to their LOD. Statistical significance was considered at $p<0.05$.

Results

Precision profiles were established for the recently introduced hs-cTnT and cTnI-Architect assay according to the NCCLS EP5 guideline. A panel of native human serum samples revealed a 10% CV cut-off value at 0.009 µg/L and 0.032 µg/L, respectively. The LOD of the hs-cTnT and cTnI-Architect assay was based on 10 measurements with cTn-negative serum (mean + 3 SD) and was <0.001 µg/L and 0.009 µg/L, respectively. With the cTnI-Architect assay, the concentrations of the reference population were <LOD in 97% of the subjects. In contrast, with the hs-cTnT assay most of the Gaussian distribution was visible (median 0.004 µg/L, 2.5th and 97.5th percentile <0.001 µg/L and 0.011 µg/L, respectively). The upper reference limit (the 99th percentile) was 0.016 µg/L and 0.013 µg/L for the hs-cTnT and cTnI-Architect assay, respectively. With the 4th generation cTnT assay, all values were <LOD.

Using the new generation cTn assays it was for the first time that pre-run cTn concentrations could be measured. As shown in table 1, with the standard 4th generation cTnT assay pre-run concentrations were <LOD in all runners and in 97% of the runners with the cTnI-Immulite. With the pre-commercial hs-cTnT assay, pre-run concentrations did not significantly differ from the concentrations in the reference popula-

Table 1. Measurements from the marathon study population in serum samples taken prior to the run, <1 h post-run and the day after the run

		cTnT 4th gen. (µg/L)	hs-cTnT (µg/L)	cTnI-Architect (µg/L)	cTnI-Immulite (µg/L)	NT-proBNP (pmol/L)	CK (U/L)	cystatin C (mg/L)	creatinine (µmol/L)
Analytical assay aspects									
	LOD	<0.01	<0.001	0.009	<0.2	0.6	<1	0.005	<1
	10% CV cut-off	0.03	0.009	0.032	0.7	n.a.	n.a.	n.a.	n.a.
	99th percentile/ref. range	<0.01	0.016	0.013	<0.2	33	M<225 F<160	0.53-0.95	M 60-115 F 50-100
Marathon population									
pre n=85	median	<0.01	0.004	0.003 [a]	<0.2	2.29	117	0.68 [b]	82 [b]
	97.5th perc.	<0.01	0.010	0.022 [a]	<0.2	15.2	293	0.90 [b]	105 [b]
	% of runners ≤LOD	100	0	82	97	13	0	0	0
	% of runners >10% CV	0	6	2	0	n.a.	n.a.	n.a.	n.a.
	% of runners >99th perc.	0	1	12	3	0	M 14 F 0	1	M 2 F 0
post <1 h n=85	median	0.01	0.029	0.026	<0.2	9.67	342	0.83 [c]	110 [d]
	97.5th perc.	0.11	0.121	0.216	0.61	43.0	2063	1.18 [c]	167 [d]
	% of runners ≤LOD	59	0	9	77	0	0	0	0
	% of runners >10% CV	19	99	45	1	n.a.	n.a.	n.a.	n.a.
	% of runners >99th perc.	41	86	80	19	5	M 83 F 87	27	M 42 F 50
post 1 d n=23	median	<0.01	0.010	0.012	<0.2	9.80	1411	n.d.	n.d.
	95-96th perc.[e]	<0.01	0.022	0.160	0.245	24.6	6253		
	% of runners ≤LOD	100	0	43	91	0	0		
	% of runners >10% CV	0	57	17	0	n.a.	n.a.		
	% of runners >99th perc.	0	30	43	9	4	M 100 F 100		

[a] measured for a selected group of n=35; [b] measured for a selected group of n=69; [c] measured for a selected group of n=51; [d] measured for a selected group of n=57; [e] because of the small number n=23, the 95-96th percentile is shown in stead of the 97.5th percentile
M, males; F, females; n.a., not available; n.d., not determined

tion (p=0.282). In contrast, with the cTnI-Architect assay pre-run concentrations were significantly higher than the reference values (p<0.001). However, pre-run levels obtained with the cTnI-Architect assay should be considered with care as 82% was <LOD. After marathon running, cTn concentrations were above the 99th percentile in almost all runners (86%) with the hs-cTnT assay. In contrast, this was true in less than half of the runners with the cTnT assay (41%) and cTnI-Immulite assay (19%). CTn concentrations obtained with the cTnI-Architect assay seemed to be comparable to the hs-cTnT assay (80%). However, only 45% of the runners exceeded the 10% CV cut-off with the cTnI-Architect assay, while with the hs-cTnT assay this was true for all concentrations (99%). The day after the run, cTn concentrations returned to <LOD with the less sensitive cTnT and cTnI-Immulite assays. In contrast, with the more sensitive assays, cTn concentrations were still increased as compared to the pre-run concentrations (p<0.001). CTn concentrations were above the 99th percentile cut-off concentration in 30% and 43% of the runners with the hs-cTnT and cTnI-Architect, respectively.

NT-proBNP pre-run levels were within the reference range (<33 pmol/L). After the marathon, NT-proBNP concentrations were significantly increased (p<0.001) but only in 5% of the runners, as shown in the table 1. Pre-run NT-proBNP concentrations correlated only weakly with cTnT in males (r=0.345, p=0.003). No correlation was found between NT-proBNP and cTn in females and between post-run NT-proBNP and cTn concentrations.

Pre-run creatinine and cystatin C concentrations were almost all within the reference range (creatinine: 98% males, 100% females; cystatin C: 99%). After marathon running, creatinine and cystatin C concentrations were significantly increased (p<0.001). Creatinine concentrations were elevated in 42% of the males and in 50% of the females, while cystatin C was elevated in only 27% of the runners. No correlation was found between post-run cystatin C and cTnT, cTnI or NT-proBNP concentrations.

Conclusions

The most sensitive cTn assay tested in the present study is the hs-cTnT assay. This assay achieved an almost Gaussian distribution in the reference population and sufficient precision in the lower concentration range (CV <10% at the 99th percentile of the reference population). The number of subjects with elevated cTn after marathon running was highly depended on the assay sensitivity. The new generation assays showed elevated cTnT and cTnI concentrations in 86% and 80% of the runners, respectively. Since clinical symptoms of a myocardial infarction were absent, there seems to be no rationale to examine all runners with positive

post-run cTn concentrations. Post-run cTn concentrations did not correlate with NT-proBNP concentrations. Furthermore, post-run cystatin C levels were increased in 27% of the runners. The number of subjects with elevated post-run creatinine levels was 1.6 to 1.8 times higher than for cystatin C, as creatinine results could be influenced by physical activity. Cystatin C concentrations did not correlate with cTn and would thereby suggest that exercise-induced cTn elevation is not caused by a reduced renal clearance. Future research is required in order to explain exercise-induced cTn elevations.

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The occurrence of CFTR mutations in patients with bronchiectasis

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Introduction

Homozygosity or compound heterozygosity for mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene causes cystic fibrosis. In Western European countries approximately 1 in 30 people is a carrier of a disease causing CFTR mutation. Carriership of such mutations is known to be harmless, however, recent data have shown that carriership might have health implications in later life, especially concerning lung function and chronicity of lung infections. Bronchiectasis is a lung disease that causes abnormal stretching and enlargement of the bronchi and is often found to be idiopathic. Recent advantages in lung imaging facilitate the diagnosis of bronchiectasis, however this did not lead to the expected increase in the number of patients. On the contrary, it was found that the occurrence of bronchiectasis has dropped significantly in the last decades. The decrease might be due to the successful treatment of lung infections with antibiotics and current vaccination programs preventing such infections. Nowadays, it is thought that the cause of the disease in the remaining patients is of a congenital nature.

A gene likely to be involved in the development of bronchiectasis is CFTR, because it encodes the transmembrane chloride channel between the epithelial cell and the lumen. A low chloride level causes extremely viscous mucus, which in turn causes clogging of the airways and predisposes to lung infections. Healthy individuals carry two correct copies of the gene, while Cystic Fibrosis (CF) patients have two mutated, dysfunctional alleles. It is believed that one working copy of the gene is enough to function normally.

Contradictory results have been published on the occurrence of CFTR mutations in patients with bronchiectasis. Several papers have shown that an increase of mutations was found, up to 60%, while others have found 0 to 4% (which does not deviate from the expected population frequency of 3.3%) (1, 2). However, in many reports, especially those yielding high numbers of mutations, patients with adult-onset CF were included in the analysis. We have started a study investigating 36 CFTR mutations and the polythymidine tract variation in adult patients with bronchiectasis of an idiopathic nature.

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