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Age-related differences in hs-cTnI concentration in healthy adults

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ABSTRACT

Background: Because the 99th percentile is of such importance in defining myocardial injury and myocardial infarction, it is important to know whether there are real age-related differences in troponin 99th percentiles.
Methods: We went to our database from the Canberra Heart Study where 1062 apparently healthy subjects were extensively screened for occult cardiac disease, and looking at persons aged < 65 years and > 65 years, for men and women separately, we compared a variety of cutpoints from the 99th percentile down to the 50th percentile.
Results: With our rigorous criteria for defining cardiac health, we excluded 67.2% of males aged > 65 years and 53.8% of women aged 65 years and older. Even with these rigorous exclusions we found that at every cutpoint examined between the 99th percentile and the 50th percentile, persons aged < 65 years had lower troponin I concentrations that persons aged 65 years and older. Similarly, at every cutpoint examined, women had lower troponin I concentrations than did men.

For the 4 separate groups examined (men and women, age < 65 years and 65 years and older) after the exclusions of persons with subclinical cardiac disease, the distributions were not significantly different to a Gaussian distribution.

Conclusions: With the rigorous exclusions of persons with subclinical cardiac disease, and the fact that our populations have a Gaussian distribution, our data suggests that age-related hs-cTnI concentrations are real. This has important implications particularly when assessing older persons in the Emergency Department.

1. Introduction

Cardiac troponin is an essential component in the diagnosis of myocardial injury and myocardial injury. The Fourth Universal Definition of Myocardial Infarction requires a rise and fall in cardiac troponin, with at least one troponin level above the 99th percentile [1].

With the recent advent of high-sensitivity troponin, most persons including children have detectable troponin in their blood [2–4]. A number of studies have shown that older persons, even when careful health screening has been performed, invariably have higher 99th percentile cut-points than do younger persons [4–7].

We hypothesised that this apparent age-related difference might be an artefact. Because the 99th percentile is such a high cutpoint, if even one or two persons with pathology were not excluded, the 99th percentile would appear much higher. If lower cut-points were used this apparent age-related difference might disappear or at least be reduced.

Using data from a community-based cohort that had been extensively assessed for the presence of cardiovascular disease [4,8], we stratified subjects on the basis of age and sex, assessed the distribution of hs-cTnI in each group and evaluated the effect of using progressively lower cutpoints to investigate whether there was convergence of decision points between persons of different age groups.

2. Methods

This study was approved by the Human Research Ethics Committees of ACT Health and The Australian National University. All subjects provided informed written consent.

2.1. Study population

Subjects for the Canberra Heart Study were originally recruited in 2003. This was a population-based study of randomly selected Canberra residents aged 60–85 years. Those who were alive and not institutionalised since the baseline visit, had a follow-up visit during 2007–2009 when subjects were aged 65–92 years, with a response rate of 93%. Serum samples used in this study were collected at this time from all 707 subjects. A further younger cohort of 355 population-based

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subjects aged 48–52 years was also recruited to the study, over the same time period as the return visit of the older cohort [9]. Of this population of 1062 subjects, 1027 had hs-cTnI assay analysis and 1001 were deemed useable with all laboratory and clinical parameters measured. Three further subjects were excluded using the outlier exclusion criteria of Dixon [10].

From this population, subjects were excluded on a variety of objective criteria. Self-reporting or identification on study assessments of any form of cardiac disease (systemic hypertension, coronary artery disease, atrial fibrillation or heart failure) resulted in exclusion. Any objective evidence of abnormalities on echocardiography that included extensive Doppler evaluation of diastolic function likewise led to exclusion. Hyperlipidemia or a fasting blood glucose ≥7.0 mmol/L or use of insulin or oral hypoglycemic agents were other clinical indications for exclusion.

Samples were collected into gel separator tubes without anticoagulant, and processed within 4 h, before freezing at −80C. Samples used in this study were subjected to a single freeze-thaw cycle and centrifuged for 10 min at 10,000*g* for 10 min before assaying. The stability of cTnI under these conditions has been demonstrated by the authors and others [11].

For this analysis, we separated subjects into those < 65 years of age and those 65 years of age and over. Two groups of subjects were studied. The first had no coning except for when the subjects sample was deemed unsuitable prior to any analysis. The second group was of those subjects remaining after both objective assessment of renal function (eGFR < 60 mL/min/1.73m^2) and heart function based on NT-proBNP levels [12]; clinical history and assessment; and transthoracic echocardiography [13] were used for exclusion [4]. Outlier exclusion using the Dixon method was also used after these other exclusions criteria were undertaken [10].

2.2. Analyses and statistical handling

The analysis of cTnI was performed on the Abbott ci16200 analyzer using the Abbott ARCHITECT STAT hs-cTnI assay, with the following analytical characteristics: LoD at 1.0 ng/L, 20% CV at 1.8 ng/L and a 10% CV at 3.9 ng/L [14].

NT-proBNP was measured on a Roche E411 analyzer with total CV of 6.6% at 124 ng/L and 7.1% at 3900 ng/L [4].

Creatinine was measured on an Abbott ci16200 analyzer and the eGFR was calculated according to the CKD-EPI formula [15]. We used an eGFR of < 60 mL/min/1.73 m2 to identify persons with stage 3 kidney disease.

Non-parametric statistical analysis was performed using Analyse-it v2.2 for Microsoft Excel.

3. Results

In Table 1, we show the relationship of age and hs-cTnI in apparently healthy, community-dwelling men and women, before exclusions were made on the basis of objective evidence of subclinical disease affecting the myocardium. At all decision points examined, men had higher hs-cTnI concentrations than did women, and for both men and women at all decision points examined the older age group had substantially higher hs-cTnI concentration.

Table 1
The relationship between age and various cutpoints for hs-cTnI in apparently healthy men and women.

hs-cTnI (ng/L)	Men		Women	
	< 65 yrs	> 65 yrs	< 65 yrs	> 65 yrs
n	157	320	164	366
99th percentile	18.5	54.8	8.3	19.1
97.5th percentile	9.5	30.8	6.1	14.0
95th percentile	7.2	17.3	4.5	10.0
90th percentile	6.0	12.5	3.4	7.3
75th percentile	3.6	6.8	2.7	4.5
50th percentile	2.8	4.2	2.2	3.1

Table 2
The relationship between age and various cutpoints for hs-cTnI in men and women who have undergone rigorous screening to remove persons with subclinical disease.

hs-cTnI (ng/L)	Men		Women	
	< 65 yrs	> 65 yrs	< 65 yrs	> 65 yrs
n	125	103	137	168
99th percentile	9.8	10.9	6.2	10.8
97.5th percentile	7.6	9.7	4.7	9.5
95th percentile	5.9	7.5	4.2	7.5
90th percentile	4.4	6.6	3.3	5.0
75th percentile	3.3	4.4	2.7	3.8
50th percentile	2.6	3.3	2.2	2.7

In Table 2, we show the relationship of age and hs-cTnI concentration in healthy community-dwelling men and women, after careful exclusion on objective grounds of persons with subclinical disease potentially affecting the myocardium. Our objective exclusion criteria resulted in 20.4% of males under the age of 65 years being excluded and 67.2% of those 65 years and over being excluded. For women, 16.5% aged < 65 years were excluded and 53.8% of those aged 65 years and over were excluded. In our objectively healthy subgroup, again, men had higher hs-cTnI concentrations than did women. At all decision points examined, there was a distinct effect of age with the younger age group having lower hs-cTnI concentrations than the older.

In Table 3, we assessed the distribution of hs-cTnI in men and women, before and after individuals were excluded on the basis of subclinical disease. Before exclusion, men and women in both age groups had distributions that were significantly different to a Gaussian population. After exclusions, for both men and women both in the younger and older age groups, populations were not significantly

Table 3
Significance testing for Gaussian distribution in coned and unconed population.

	Test for Normal distribution	Tests for significance	
	Kolmogarov-Smirnov (p)	ANOVA	Mann-Whitney
		Probability (p)	Probability (p)
Unconed (before exclusion)			
Male			
< 65 yrs	< 0.01		
> 65 yrs	< 0.01		
< 65 yrs. v > 65 yrs		0.21	< 0.01
Female			
< 65 yrs	< 0.01		
> 65 yrs	< 0.01		
< 65 yrs. v > 65 yrs		< 0.01	< 0.01
Coned (after exclusion)			
Male			
< 65 yrs	> 0.10		
> 65 yrs	> 0.05		
< 65 yrs. v > 65 yrs		< 0.01	< 0.01
Female			
< 65 yrs	> 0.15		
> 65 yrs	> 0.15		
< 65 yrs. v > 65 yrs		< 0.01	< 0.01

different to a Gaussian distribution, but the younger and older age groups maintained a significantly different hs-cTnI concentration distribution.

4. Discussion

It has been noted in the literature that older persons have higher cTn concentrations than do younger persons, in both population studies [4–7,16–24] and clinical studies of persons presenting to the Emergency Department [25–27]. However, these studies were heterogeneous with regard to exclusion of subjects with possible subclinical cardiac disease.

The Canberra Heart Study has been the most rigorous with strict exclusion criteria being used to define a healthy population after coning. A history of systemic hypertension, hypercholesterolemia, diabetes mellitus, atrial fibrillation, coronary artery disease, heart failure or use of vasoactive medications or diabetic drugs (insulin or oral hypoglycemic agents) were exclusions. All subjects underwent 2-dimensional and Doppler echocardiography and reduced left ventricular ejection fraction below 50%, abnormal relaxation filling pattern, increased cardiac filling pressures, increased indexed LV mass and valvular heart disease were all grounds for exclusion. Further grounds for exclusion based on laboratory criteria included a fasting glucose ≥ 7.0 mmol/L, an eGFR < 60 mL/min/1.73m^2, or increased NT-proBNP concentration [4,12]. No other studies have been as rigorous in their exclusion criteria and the fact that the 4 groups (men and women $<$ 65 years and 65 years and over) all displayed a Gaussian distribution is supportive of our claim that these persons were truly healthy.

In our analysis of data from the Canberra Heart Study, we have found there is a distinct relationship between age and cTnI concentration in healthy persons right down to 50th percentile. Thus our initial hypothesis – that the age association was artifactual due to underlying subclinical cardiac disease or to the statistical problem of extreme outliers at the 99th percentile – appears to be incorrect and there is a real age association independent of disease.

Eggers et al. [7] have reported similar findings. However, they did not perform echocardiography on all their subjects and they had many fewer exclusions than we did suggesting that subclinical disease may still have been present. In addition, they reported combined data without stratification for gender. In light of the clear effect of sex on underlying cTn concentrations in healthy persons [4], this further confounded the findings.

Despite the rigorous screening for cardiac structural disease with echocardiography and heart failure using clinical and biomarker assessments, it is possible that age-related myocardial dysfunction has not been identified in this study. Although it is still recommended as the standard measure of systolic function [28], left ventricular ejection fraction is a less sensitive marker of impaired systolic function than global longitudinal strain [29], with evidence that troponin levels are better correlated with the latter index [30]. Furthermore, there is increasing evidence from clinical trials of heart failure with preserved ejection fraction [HFpEF], that the syndrome of heart failure can be prevalent without increased left ventricular mass in up to 50% of patients or Doppler evidence of even mild diastolic dysfunction in up to 30% [31]. These findings have led to the awareness of the heterogeneity of biological phenotypes for HFpEF, with evidence of increased levels of troponin with coronary microvascular dysfunction [32] and structural and functional myocyte abnormalities related to titin isoform shifts, mitochondrial dysfunction and abnormal calcium handling [33]. Accordingly, it is possible that increased levels of troponin at the 99th percentile in older persons may still be a reflection of age-related myocardial dysfunction that could not be measured using conventional methods. Against this is that each of our populations had a distribution not significantly different to a Gaussian population.

The major limitation of our study is that there were small numbers remaining in the older age-group after exclusion of persons with objective evidence of cardiac disease. Considering we started with a population of $>$ 1000 subjects, this will always be a problem when this particular topic is addressed. A further limitation was that we were unable to assess HbA1c as no sample was available.

In conclusion, the discussion regarding age related cTn cutpoints has been ongoing for many years and there has been calling for more studies in the area [34]. We have demonstrated that in healthy persons there are higher troponin concentration in older persons, not just at the 99th percentile but right down to the 50th percentile. This has important implications, particularly when assessing patients with possible ACS in the Emergency Department.

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