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Title: **High-sensitive cardiac troponin hs-TnT levels in sudden deaths related to atherosclerotic coronary artery disease.**

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Highlights

- There is a significant association between hs-TnT serum values and the presence of ischemic heart disease as the cause of death
- Cardiopulmonary resuscitation does not increase the hs-TnT levels
- hs-TnT level increases in post-mortem, both in cases and controls
- More studies on cardiac biomarkers are needed in postmortem practice

ABSTRACT

INTRODUCTION: Ischemic heart disease (IHD) related to atherosclerotic coronary artery disease (CAD) is one of the most prevalent causes of death in Europe. Postmortem evaluation of IHD remains a challenge because of possible non-specific autopsy finding in some autopsy cases, especially in early myocardial ischemia. High-sensitive cardiac troponin T (hs-TnT) is used today in clinical practice as the "gold standard" to diagnose the myocardial ischemia, and might also be applied as an ancillary tool for post-mortem evaluation.

PURPOSE: The goal of this study is to evaluate the diagnostic value of post-mortem serum hs-TnT assay in cases of sudden death related to IHD. We will also investigate the influence of cardiopulmonary resuscitation (CPR) attempts on post-mortem hs-TnT levels.

METHODS: The hs-TnT values in serum were retrospectively analysed in 85 autopsy data. 52 cases with clinical history and morphological results suggesting cardiac ischemia were included in the study group (mean age 53.5; age range 34-75) and 33 cases in the control group (mean age 40.4; age range 15-69). The group's statistical comparison was performed using logistic regression model.

RESULTS: Our study showed a significant non-linear association between hs-TnT serum values and post-mortem diagnosis of sudden deaths related to IHD (*p*-value 0.005). The shape of the relationship is showing that the probability of death due to IHD increases quickly with a light level of hs-TnT (maximum around 90ng/L) then decreases slightly while remaining at

high in values. No significant difference in the hs-TnT serum values was found between the CPR and the non-CPR cases (*p*-value 0.304).

CONCLUSION: The measurement of hs-TnT serum values might be considered as an ancillary tool for the evaluation of death related to IHD, while taking necessary precautions in the interpretation of the results.

Key words: High-sensitive cardiac troponin hs-TnT, sudden death, myocardial ischemia

INTRODUCTION

One of the most prevalent causes of death in Europe is ischemic heart disease (IHD) related to atherosclerotic coronary artery disease [1]. In clinical medicine, the diagnosis of IHD is based on clinical symptoms, electrocardiogram and biochemical markers. Post-mortem evaluation is more complicated as the anamnesis and diagnostic method are limited. Moreover, autopsy findings are not always specific for sudden death related to IHD, particularly if arrhythmias follow an acute coronary event or after a resuscitation attempt [2]. Forensic pathologists have turned towards the use of cardiac biomarkers initially developed in clinical medicine in an attempt to facilitate diagnosis of post-mortem IHD. These markers could become useful when morphological results are not conclusive at the autopsy.

Among biomarkers, cardiac troponin (cTn) is the most frequently used in clinical practice as more sensitive and specific marker of cardiomyocyte injury than other biomarkers [3]. The troponin is a protein complex released in the blood flow after a myocardial injury. The clinical measurements of troponin appeared first in the mid-2000s and is considered by clinicians as the "gold standard" for acute myocardial infarction (AMI) diagnosis [4]. Last years, the old cTn measurement was replaced by the high-sensitive cardiac troponin T (hs-TnT) assay, using the same immunoassay method with new antibodies, more sensitive, able to detect AMI at an earlier time-point than old cTn tests. AMI can be reliably identified within 3 h after admission with up to 100% sensitivity and up to 100% negative predictive value using hs-TnT assay, indicating that observation time may be reduced for the rule-out and rule-in of AMI, with various clinical algorithms [4-6]. Today, the hs-TnT assay is the "gold standard" for AMI diagnosis in clinical practice [4,5,7]. It is however important to notice that the

increase of the level of troponin reflects the myocardial cells' necrosis and is thus not specific [5].

Many clinical cardiac biomarkers were tested in post-mortem examination [8-22]. The post-mortem experimental use of the old cTn assay started early already in the 2000s. The added value of its studies outcomes in forensic diagnosis always remained controversial, especially concerning the cause of death. There are also different opinions about the effects of cardiopulmonary resuscitation (CPR) on the increase of cTn level. Some authors suggested that the potential cardiac trauma could affect the release of troponin [23], however the scientific literature reports mainly that the effects on troponin level were non-significant in post-mortem [10,20,24]. To date, only one study has been driven using the new hs-TnT assay on postmortem serum and pericardial fluid by González-Herrera et al. in 2016 [25] but did not focus on IHD. The authors showed that serum levels do not show any correlation with any cause of death and that low hs-TnT levels in pericardial fluid allowed the exclusion of the heart damage.

Then, although many studies concerned the evaluation of biomarkers in postmortem, literature remains rather uncertain about the post-mortem diagnosis value of hs-TnT. Points of view emerged that troponin could be useful as an ancillary diagnostic tool to identify the cause of death, while one must remain cautious when interpreting the results. Therefore, this new biomarker requires more post-mortem studies. The goal of our study is therefore to evaluate the diagnostic value of post-mortem hs-TnT's assays. We will focus on IHD's sudden death cases related to atherosclerotic coronary artery disease, with or without morphological signs of cardiac ischemia. The influence of CPR attempts on post-mortem hs-TnT levels will also be evaluated.

MATERIAL AND METHODS

Cases

The retrospective study was performed on autopsy data retrieved from the database of autopsies conducted by the University Center of Legal Medicine in Lausanne (CURML) between 2012 and 2015. The estimated post-mortem period did not exceed 72 hours. Study cases were selected on the basis of clinical history and/or morphological results suggesting the death from cardiac ischemia; this included cases with an acute coronary thrombosis or with signs of an acute myocardial ischemia, cases with coronary stenosis > 75 % with or without signs of an acute myocardial ischemia and without other cause of death found at autopsy and during toxicological analyses. Control cases included cases of violent death as hanging, strangulation, extra thoracic trauma, without an extended agony and without clinical or autopsy findings suggesting any cardiovascular disease.

Considering that other causes than IHD, cardiac or not, can lead to an increase of hs-TnT as myocarditis, pericarditis, different kinds of heart failure, chronic renal failure, cerebrovascular accidents, acute pulmonary embolism, chronic obstructive pulmonary disease, drug intoxication and other acute non-cardiac critical illness [25,26,5], such cases were excluded from the study. Exclusion criteria included also advanced cadaveric deterioration and postoperative death. Person who had previously refused in a written document to give his/her corporal substance for research use were excluded.

In each case, the examination of the heart involved macroscopical and histological evaluations. The histological examination was performed including at least five different topographic locations; namely left ventricle (anterior, lateral, posterior), interventricular septum and lateral wall of the right ventricle. The sections were routinely stained with

haematoxylin–eosin, followed in selected cases by immunochemistry (fibronectin and C5b-9). The body and heart weights were recorded for each case.

This study received the ethical approval by the Cantonal Commission for the Ethics of Human Research (CER-VD).

Biochemical analyses

Peripheral blood from the femoral veins was systematically collected as soon as possible upon arrival of the bodies at the morgue and prior to autopsy. Blood was collected by aspiration with sterile needles and syringes from the femoral vein(s). Blood samples were drawn after clamping the vein(s) at the proximal end and lifting the lower limb(s) for several minutes. Samples were stored in preservative free gel serum separator tubes, that were centrifuged immediately post collection at 3000g for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in preservative free tubes. Postmortem serum samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at - 20°C.

Levels of postmortem serum cardiac troponin T were measured with hs-TnT reagents by electrochemiluminescence immunoassay (ECLIA). Results were expressed in nanograms per liter [19]. Cases with the troponin values higher than 10’000ng/L were excluded as considered as excessively altered and outside the analytical range.

Statistical analyses

Categorical data were summarized by their frequencies and percentages. For the age, the body weight and the hearth weight, the summary was given by their mean (sd) and range. Univariate logistic regression was performed to assess the association between each predictor and the outcome “cause of death” (reported as Odds-Ratios (OR) and p-values). The

functional form of the relationship between the continuous variables (hs-TnT levels, age, body weight and hearth weight) and the probability of death due to IHD was checked using a fractional polynomial model. The linearity assumption was confirmed except for the troponin T (hs-TnT) level (Figure 3). The level of the troponin T (hs-TnT) was then coded into three categories (≤12 coded by 0, [13-2250] coded by 1 and ≥ 2251 coded by 2) to assess the risk of death due to IHD when being in the categories 1 or 2 compared to the reference category 0.

RESULTS

A total of 85 cases (68 males and 17 females) were selected, 52 for the study group (age range 34-75) and 33 for the control group (age range 15-69). Among those from the study group, acute thrombosis was found in 19 cases, 23 cases presented a severe atherosclerosis (with >75% of luminal stenosis) without coronary thrombosis and without other cause of death found at autopsy and during toxicological analyses and finally 10 cases had coronary luminal stenosis 50-75% and signs of an acute myocardial ischemia confirmed by immunochemistry and without other cause of death found at autopsy and during toxicological analyses. The control group included 23 cases of hangings or other asphyxia and 10 traumatic deaths. The manual CRP was performed in 28 cases from the study group and in 15 control cases.

The troponin levels varied between 3 and 9687 ng/L. Complementary characteristics about cases and control are summarized in Table 1 and data related to coronary lesions in Table 2.

Statistical results

No statistical correlation with hs-TnT values were found considering the time period between the death and the serum sample analysis measured for both cases and controls (Fig 1). There were also no significant differences between hs-TnT values of reanimated and non-reanimated cases from the control group (*p*-value 0.304; Fig. 2). Therefore, samples of cases and controls were taken without taking those characteristics into account.

A non-linear significant association between hs-TnT serum values and the presence of IHD (p-value 0.005. Fig.3) was observed. The shape of the relationship is showing that the probability of death due to IHD increases quickly with a light level of hs-TnT (maximum around 90ng/L), then decreases slightly while remaining at a high level.

It was also noticed that the increase of hs-TnT levels in elderly patients with heavy heart weight, in presence of atherosclerosis or of cardiovascular symptoms before death (prodome), were significantly associated with a high probability of being included in the “study case” category.

DISCUSSION

The use of clinical medicine biomarkers is a challenge for the forensic pathologists. While their interpretation is established in a clinical context, it is not far the case for post-mortem use. The actual challenge when using post-mortem biomarkers lies in the interpretation of these values that need to be adapted to the context of post mortem evaluation.

The values of hs-TnT observed in this study and their interpretation are different from the ones seen in clinical medicine. The results of this study demonstrate that the hs-TnT level increases in post-mortem, both in cases and controls. This fact is often mentioned in the literature. Thus, regardless of the technique used (TnI, TnT, hs-TnT), levels of cardiac biomarkers are usually significantly higher than the reference range for living patients [27,28,25,21,22]. The reason for this upsurge is unclear; haemolysis and autolysis were suggested [28,29], as well as non-specific lesions and "invisible" infarction due to hypoxia of the myocardium during terminal agony [21,22]. It seems thus clear that the references values used clinically (ante-mortem) could be meaningless or questionable in the post-mortem context.

This study revealed a non-linear significant association between hs-TnT serum values and the presence of IHD. This result differ from the findings of the González-Herrera et al. study, reporting no correlation between the different causes of death and the serum levels of cTnT [25]. An increase of probability of mortality from the IHD group with an increase of hs-TnT level was noticed, with a peak value around 90 ng/L. Above this value, the probability does not increase anymore, on the contrary to the use of hs-TnT in clinical medicine [4]. Moreover, beyond this limit, a slight decrease of the probability of IHD was observed. Therefore,

according to the results of our study, very high values of hs-TnT (as 2500 ng/L) were not correlated with a higher probability of IHD-related death. It was observed also that the correlation between the increase of hs-TnT and the probability of IHD death was higher in older victims, with an increased weight of heart, and atherosclerosis and cardiovascular symptoms before death (prodromes). Those results are not surprising and are in accordance with literature, clinical data as well as with physiopathology of the myocardial ischemia [30].

This study showed that the attempt of a CPR does not seem to lead to a significant increase of post-mortem hs-TnT. These findings are in accordance with the results found in scientific literature [10,20,31] concerning the old troponine assay. Also, clinical studies about the hs-TnT support that CPR may lead to a non-negligible increase of troponin in survivors [23]. No other evaluation was reported to this day for post-mortem evaluation of hs-TnT in reanimated cases.

Post-mortem troponine levels often depend on sampling sites. It was reported that pericardial fluid would be the most representative sample site (more direct “salting out” of troponin from the myocardium to the sample site due to the proximity of each other) [28,21,22]. The troponin in pericardial fluid seems to show a better negative predictive value for post-mortem investigations than serum. This has been raised regarding the old troponin assay [14,16,17], as well as in the single post-mortem evaluation of hs-TnT assay [25]. The hs-TnT levels found in this study were measured only in serum samples and the evaluation of the hs-TnT levels in pericardial fluid for the post-mortem diagnosis of IHD would be interesting in future studies.

Finally, it should also be underlined that, in a clinical context, it has recently been demonstrated that the specificity of hs-TnT for acute myocardial infarction is very low as the

increased values are observed in myocardial damage of any origin [26,32]. Therefore, the interpretation of a rise of the hs-TnT should be very careful and that more studies with larger samples could be useful.

Study limitation

The essential limitation of this study is related to the post-mortem context, impossible to be ruled out. This could lead to a biased increase of hs-TnT as reported for other post-mortem studies. The number of cases and controls included in this study is relatively small to obtain a good significance of the results. For practical reasons, hs-TnT levels in pericardial fluid were not tested in this study. Therefore, we suggest performing more studies to further our knowledge in this interesting topic.

CONCLUSION

This study showed a non-linear significant association between hs-TnT serum values and the presence of IHD as the cause of death, with a different profile compared to the clinical field (peak around 90ng/L, then slight decrease of the probability). It was however impossible to determine a cut-off value as for living patients in clinical medicine, probably because of the non-specific and unpredictable rise of hs-TnT due to post-mortem alterations. No significant difference of hs-TnT serum values was found between the CPR and the non-CPR cases.

It seems reasonable to state that hs-TnT assay in serum can be considered as an additional tool for the evaluation of death related to IHD, while remaining cautious when interpreting the results. However, the use of this new high-sensitive assay requires more studies, especially with a focus on the pericardial fluid.

Legends

Fig. 1; Description of the variability of hs-TnT level in serum according to the post-mortem period in the two groups; cases and controls.

Fig. 2; Distribution of hs-TnT level in serum for control-cases reanimated versus not reanimated. No significant difference were noticed (two sample Wilcoxon test).

Fig. 3; Fig(a): fitted non-linear relation-ship between the hs-TnT level in serum and the predicted(logit)+residuals for death related to IHD. Fig(b): The probability of death related to IHD according to the hs-TnT level in serum. Fig(c): the same as Fig (a) with the zoom for the hs-TnT level in serum between [3-2250]. Fig(d): the same as Fig(b) with the zoom for the hs-TnT level in serum between [3-2250]. The two red vertical lines correspond to the hs-TnT level in serum=12 and 2250. Blue dashed horizontal line in Fig(d) indicate that the probability of death related to IHD was upper to 50% for the hs-TnT level in serum between [3-2250].

Table 1; Summary of data relating the main characteristics including the number, the age, the body weight, the heart weight and the hs-TnT values for cases and controls. The association between each characteristic and the outcome measured using the OR and associated *p-value*.

Table 2; Summary of data in subgroups of cases and controls. For statistical analyses, the level of the troponin T (hs-TnT) was coded into three categories ($\leq$12, [13-2250] and $\geq$ 2251) to assess the risk of death due to IHD (for details see Statistical analyses).

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Figure 1

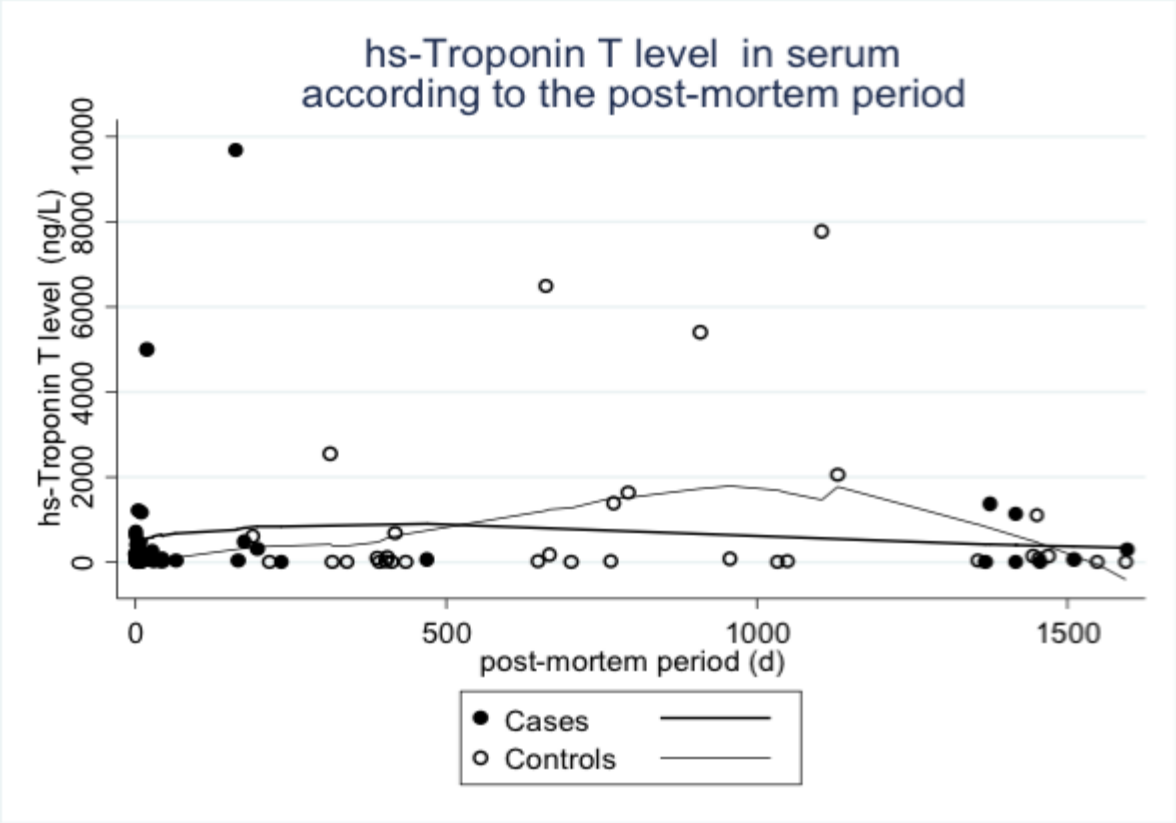

Fig.1 Description of the variability of hs-TnT level in serum (ng/L) according to the post-mortem period (day) in the two groups; cases and controls.

Figure 2

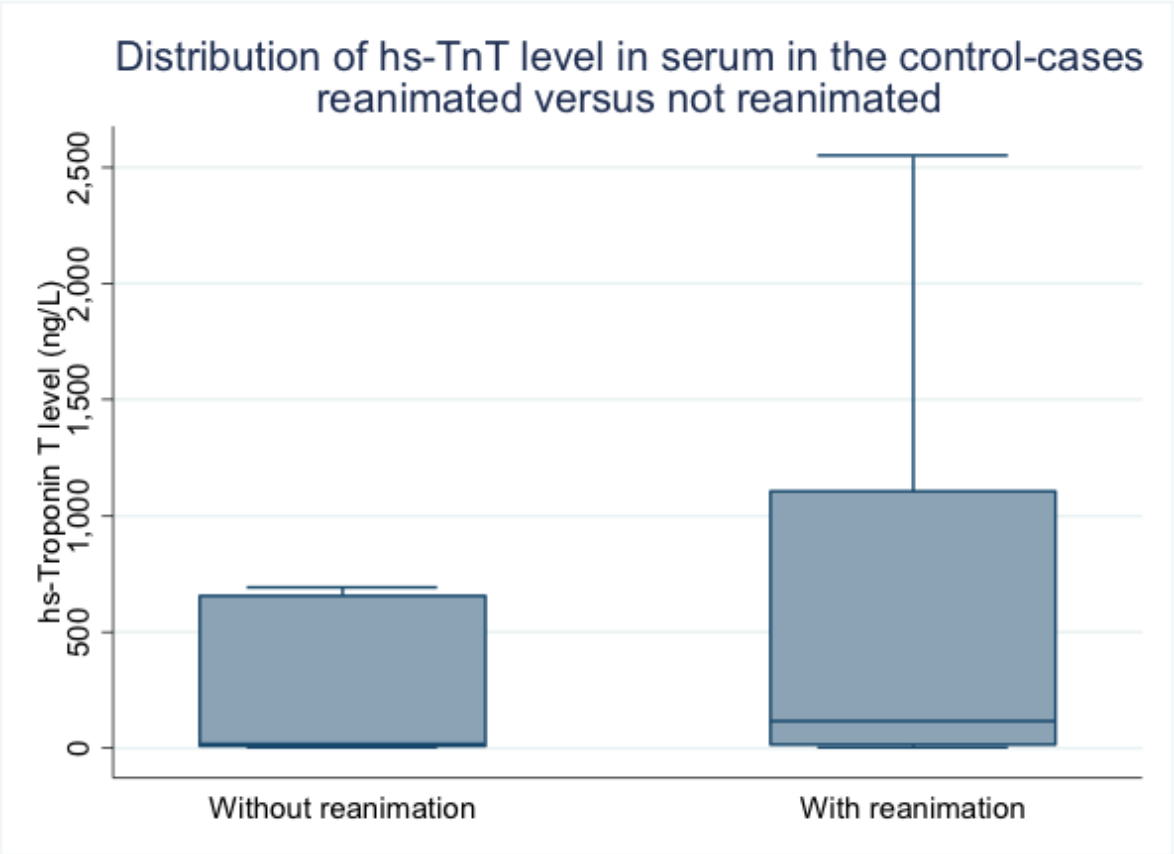

Fig. 2 Distribution of hs-TnT level in serum (ng/L) for control-cases reanimated versus not reanimated. No significant difference were noticed (two sample Wilcoxon test).

Figure 3

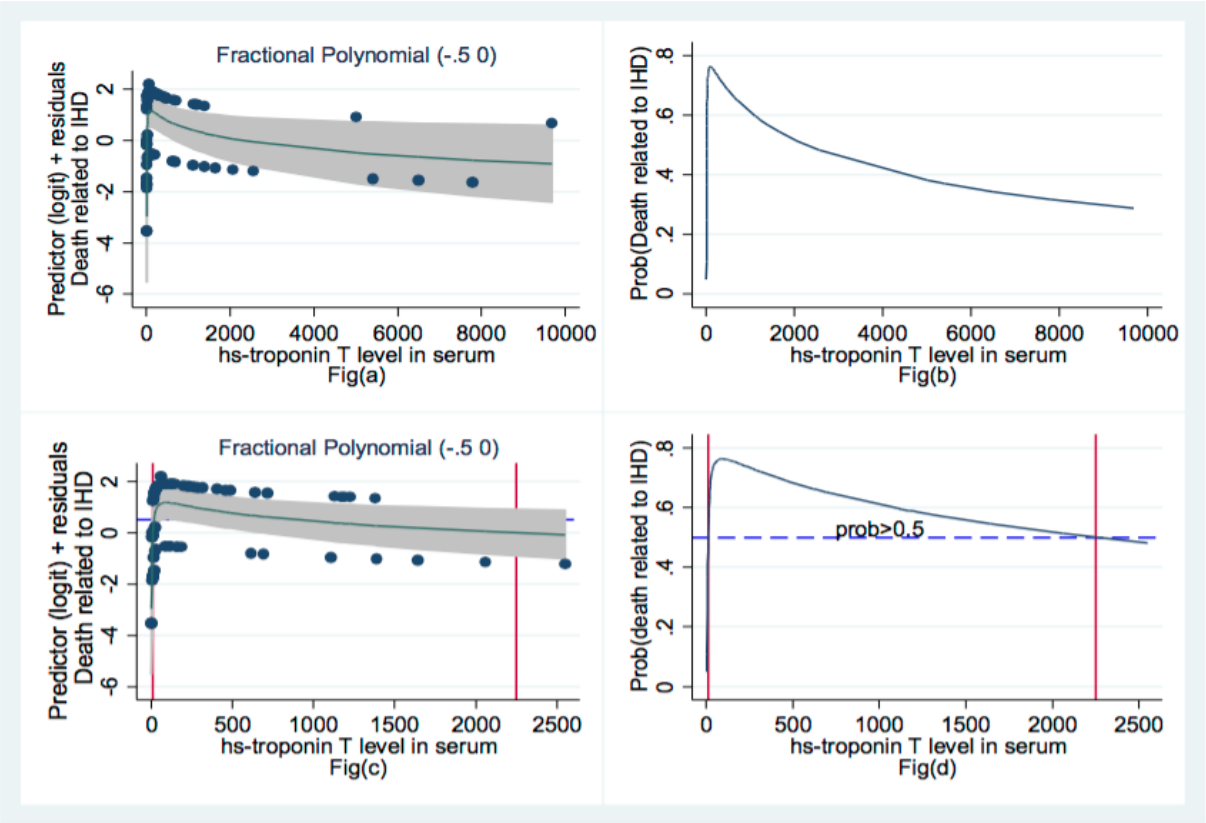

Fig. 3 Fig(a): fitted non linear relation-ship between the hs-TnT level in serum (ng/L) and the predicted(logit)+residuals for death related to IHD. Fig(b): The probability of death related to IHD according to the hs-TnT level in serum (ng/L). Fig(c): the same as Fig(a) with the zoom for the hs-TnT level in serum (ng/L) between [3-2250]. Fig(d): the same as Fig(b) with the zoom for the hs-TnT level in serum (ng/L) between [3-2250]. The two red vertical lines correspond to the hs-TnT level in serum=12 and 2250 (ng/L). Blue dashed horizontal line in Fig(d) indicate that the probability of death related to IHD was upper to 50% for the hs-TnT level in serum between [3-2250].

Table 1

Variable	Cases N (%)	Controls N (%)	Odd ratio	*p- value*
N (%)	52 (61.2)	33(38.8)		
Sex **Male, n (%)**	45 (86.5)	23 (69.7)	2.79	*0.06*
Age [y] **Mean (sd)** **min-max**	53.54 (9.5) 34-75	40.42 (14.1) 15-69	1.09	*<0.0001*
Body weight [kg] **Mean (sd)** **min-max**	82.12 (14.9) 55-140	75.36 (18) 43-121	1.03	*0.07*
Heart weight [g] **mean (sd)** **min-max**	477.50 (108.9) 285-790	350.76 (90.8) 175-565	1.01	*<0.0001*
Hs-TnT[ng/L] **≤12[ref]** **[13-2250]** **>2250**	4(7.7) 46(88.5) 2(3.8)	10(30.3) 19(57.6) 2(3.8)	- 6.05 1.25	 *0.005* *0.83*

Table 1 Summary of data relating the main characteristics including the number, the age, the body weight, the heart weight and the hs-TnT values for cases and controls. The association between each characteristic and the outcome measured using the OR and associated *p-value*.

Table 2

	Coronary lesions	Cases n	Sex Male, n(%)	Age (y) Mean (sd) min-max	Heart weight (g) Mean (sd) min-max	Lungs weight (g) Mean (sd) min-max	Hs TnT levels (ng/L)			HsTn level Mean (sd) min-max
							≤ 12	13-2250	>2250	
Cases	Acute coronary thrombosis	19	15 (78.9)	47 (7.7) 34-67	446.3 (80.9) 285-640	1834.5 (607) 600-2900	1	16	2	1128.9 (2365.7) 4-9687
	Coronary stenosis <75% with signs of myocardial ischemia	10	9 (90)	58.3 (8.9) 47-75	551 (162.5) 345-790	1711 (440.1) 1235-2350	1	9	0	63.4 (90.3) 12-317
	Coronary stenosis >75%	23	21(91.3)	56.1 (9.2) 37-70	471.3 (89) 315-650	1605.2 (377.7) 1050-2470	2	21	0	211.8 (331.7) 8-1191
	All study cases	52	45 (85.5)	53.5 (9.5) 34-75	477.5 (108.9) 285-790	1709.3 (486.3) 600-2900	4	46	2	518.3 (1498) 4-4687
Controls	None	33	23 (69.7)	40.4 (14.1) 15-69	350.8 (90.8) 175-565	1081.7 (329.4) 495-1720	10	19	2	935.5 (942.8) 3-7786

Table 2; Summary of data in subgroups of cases and controls. For statistical analyses, the level of the troponin T (hs-TnT) was coded into three categories (≤12, [13-2250] and ≥ 2251) to assess the risk of death due to IHD (for details see Statistical analyses).