A Case Series of Disproportionate Elevations of Cardiac Troponin and Macrotroponin in Fabry Disease

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Abstract

Fabry disease is a rare X-linked lysosomal storage disorder that causes progressive cellular accumulation of glycosphingolipids, leading to various end-organ manifestations such as chronic kidney disease and cardiomyopathy. Currently, troponin is the preferred biomarker to identify acute coronary syndromes and cardiac inflammation/myocarditis, as well as monitor myocardial damage. Macrotroponin is an immunoglobulin G-troponin bound complex with reduced clearance due to its higher molecular weight. This can cause false elevations in troponin, in the absence of myocardial damage, which has been reported in up to 5% of patients presenting to emergency departments in Australia. In this case series, we report on ten Fabry patients in whom macrotroponin was demonstrated after precipitation with polyethylene glycol (PEG). Of the 47 routine clinical samples of Fabry patients that were analysed, troponin was demonstrated to be elevated in 15 samples (32%), and ten of these demonstrated macrotroponin (21% of total, 67% of elevated troponin). This case series highlights the need to consider the possibility of macrotroponin in Fabry patients with elevated troponin. This relatively high prevalence raises the questions of whether Fabry patients are intrinsically more predisposed to macrotroponin and how this influences clinical management, which warrants further research.

Keywords

Cardiomyopathy, Fabry Disease, Macrotroponin, Troponin.

Introduction

Fabry disease (FD) is a rare X-linked lysosomal storage disorder caused by pathogenic variations in the *GLA* gene, which affects hemizygous males and heterozygous females [1,2]. This leads to deficiency of the lysosomal alpha-galactosidase A enzyme, which results in progressive cellular accumulation of glycosphingolipids in various organ systems, including cardiac myocytes, vascular smooth muscle cells and endothelium [1]. There are several cardiac and renal manifestations in FD, which include chronic kidney disease, left ventricular hypertrophy, cardiac fibrosis and cardiomyopathy [1,3]. The mainstay of management of FD is early diagnosis and introduction of enzyme replacement therapy (ERT) or chaperone therapy which may improve symptoms and quality of life [4,5].

The preferred biomarker in the assessment and monitoring of myocardial injury (including but not limited to cardiac inflammation/myocarditis, myocyte necrosis and acute coronary syndrome) is high-sensitivity cardiac troponin (hs-cTn) [6]. Troponin is a complex of three regulatory subunit proteins that are responsible for the contraction and relaxation of striated muscle [7]. In addition to acute coronary syndromes, raised troponin has been noted in numerous other conditions, including those associated with FD such as chronic kidney disease and cardiomyopathy [2,8]. While the current gold standard method of assessment and surveillance of cardiomyopathy in FD is imaging through cardiac magnetic resonance imaging (CMR) and/or

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echocardiography [9], hs-cTn is increasingly being recognised as a potential biomarker in the staging and follow-up of FD patients [1]. It has been postulated to be an accurate and widely accessible biomarker for the detection of inflammation and/or fibrosis in FD [10,11], and an indicator of cardiomyopathy progression [10].

However, the existence of circulating autoantibodies to troponin have been identified as a cause of artefactual increases or decreases in troponin [12]. Macrotroponin is an immunoglobulin-troponin bound complex that comprises endogenous IgG antibodies bound to either troponin I or T fragments [13]. The formation of an antibody complex may reduce the clearance of troponin which can lead to falsely elevated troponin on testing [14]. This may confound the clinical picture and prompt unnecessary and potentially harmful further investigations and interventions. In a 2016 Australian study, macrotroponin was reported in up to 5% of individuals presenting to emergency department who were found to have elevated troponin I (hs-cTnI) [15]. It has been suggested that troponin I mid-fragments are more susceptible to interference by macrotroponin than troponin T [16].

To date however, there have been no known studies that have quantified the prevalence of macrotroponin in the FD cohort, especially in those with elevated routine troponin. Given the growing importance of troponin as a biomarker for cardiac disease in FD, it is critical that the potential interference posed by macrotroponin be recognised and understood in clinical practice, to avoid unnecessary investigation and intervention.

The aim of this paper was to report on a case series of elevated troponin concentrations that were identified among routine clinical samples of FD patients. These samples were originally intended to be correlated against routine patient imaging, as per the Fabry Imaging Clinical Study. Once it was discovered that a proportion of samples had elevations in troponin, further testing was undertaken to identify macrotroponin. This paper (a) quantifies the prevalence of macrotroponin among a cohort of FD patients with elevated troponin and (b) correlates this with clinical findings.

Methods

Study Population

The study population consisted of FD patients who are known to the Genetic Metabolic service at Westmead Hospital (WMH), Sydney, Australia. As per routine biochemistry testing, patient samples were received at the pathology lab of the Institute of Clinical Pathology and Medical Research (ICPMR) at WMH. Patient electronic medical records were reviewed, to obtain basic demographic information as well as clinical information including the FD genotype, phenotype and treatment status of the patient. All patients were deemed clinically stable and all samples collected for the purpose of this study were routine and not in the context of patients presenting acutely with any cardiac symptoms. This study is part of the Fabry Imaging Clinical Study, which received ethics approval by the Western Sydney Local Health District (WSLHD) Human Research Ethics Committee (2019/ETH08330).

Troponin testing

The lithium-heparin samples received by the ICPMR laboratory at WMH were tested for troponin using the Siemens Atellica High Sensitivity Troponin-I assay on a Siemens Atellica IM 1300 analyser. This assay is the method routinely used by the ICPMR laboratory in the measurement of cardiac troponin I. All samples that registered a hs-cTnI level greater than 50 ng/L by the Atellica hs-cTnI assay were considered to be elevated, as this value represents the >99th percentile reference range specified by the lab and assay used in this study. Samples with elevated troponin were also subjected to polyethylene glycol (PEG) precipitation for the identification of macrotroponin, plus treatment with heterophile blocking tubes (HBT) (Scantibodies Laboratory, Santee, CA, USA). This was done as per manufacturer instructions, to screen for interference to the assay from heterophile antibodies.

PEG precipitation

PEG precipitation was performed for the identification of macrotroponin, using a similar method to that previously described by Warner et al. [15] A 25% solution of PEG 6000 (molecular weight) was mixed with phosphate buffered saline and the plasma sample being analysed in a 1:1 ratio. The mixture was vortexed for approximately ten seconds and incubated for ten minutes at room temperature. The mixture was then centrifuged (5 min at 14000g), before the supernatant was analysed for its hs-cTnI content, which was subsequently multiplied by the dilution factor of two and expressed as a percentage of the original plasma hs-cTnI. After precipitation with PEG, samples that recovered less than 25% of their initial hs-cTnI level were considered to indicate macrotroponin.

Results

Troponin and macrotroponin

There were 47 samples taken from 47 Fabry patients, including 26 males (55%), with a mean age of 49 years (standard deviation 15.4 years). Although the majority of these samples were transferred to or received directly by the pathology laboratory at WMH, some were analysed for hs-cTnI remotely due to rural location or local laboratory COVID-19 procedures. Of the 47 samples that were analysed in total (both remotely and at Westmead Hospital), 17 (36%) had elevated hs-cTnI levels that equalled or exceeded the 50 ng/L threshold (mean 994 ng/L; range 50 – 7809 ng/L). However, two of the 17 samples were not physically

received at WMH and therefore could not undergo further testing. For the 15 samples (32%) received by the pathology lab at WMH, interference from heterophile antibodies was excluded as incubation in HBT tubes demonstrated recoveries ranging between 90 and 119%.

After PEG precipitation of the 15 samples with elevated troponin, ten samples registered hs-cTnI recovery proportions that were below the recovery threshold of 25% (Figure 1), indicating the presence of macrotroponin. As such, the prevalence of macrotroponin was 21% among the entire FD cohort tested in the study, and 66.6% among the group with elevated hs-cTnI. Of note, mean hs-cTnI among the ten patients with macrotroponin was 1579 ng/L (standard deviation 2231 ng/L). In comparison, this exceeded the mean hs-cTnI among the remaining five patients with elevated troponin without macrotroponin (mean 159 ng/L; standard deviation 63 ng/L). The post-PEGylation hs-cTnI percentage recoveries in the patients who demonstrated macrotroponin ranged between 3.7% and 21.7%, averaging at 12.2% (standard deviation 6.9%). This is in comparison to the post-PEGylation recovery of hs-cTnI from control samples, which ranged between 37% - 134%. These control samples were independent to this study and sourced from the WMH ICPMR laboratory from non-FD patients with elevated hs-cTnI who were known not to have macrotroponin.

Clinical data

Among the 15 individuals who registered elevated hs-cTnI, nine were female (60%) and ages ranged between 48 and 79 years (mean age 60 years; standard deviation 8.1 years). The demographic and clinical characteristics of the ten patients with macrotroponin, as well as their biochemical testing results, are presented in Table 1. While the genotype varied across the ten patients, 90% were of the classical FD phenotype, and 60% were actively receiving enzyme replacement therapy. Renal function varied across the group, with half the patients having intact renal function and the other half having mild to moderate renal impairment. Of note, in our small cohort, we observed a higher female preponderance among the group with macrotroponin (80% female), in comparison to the group with lower-level elevations without macrotroponin (20% female).

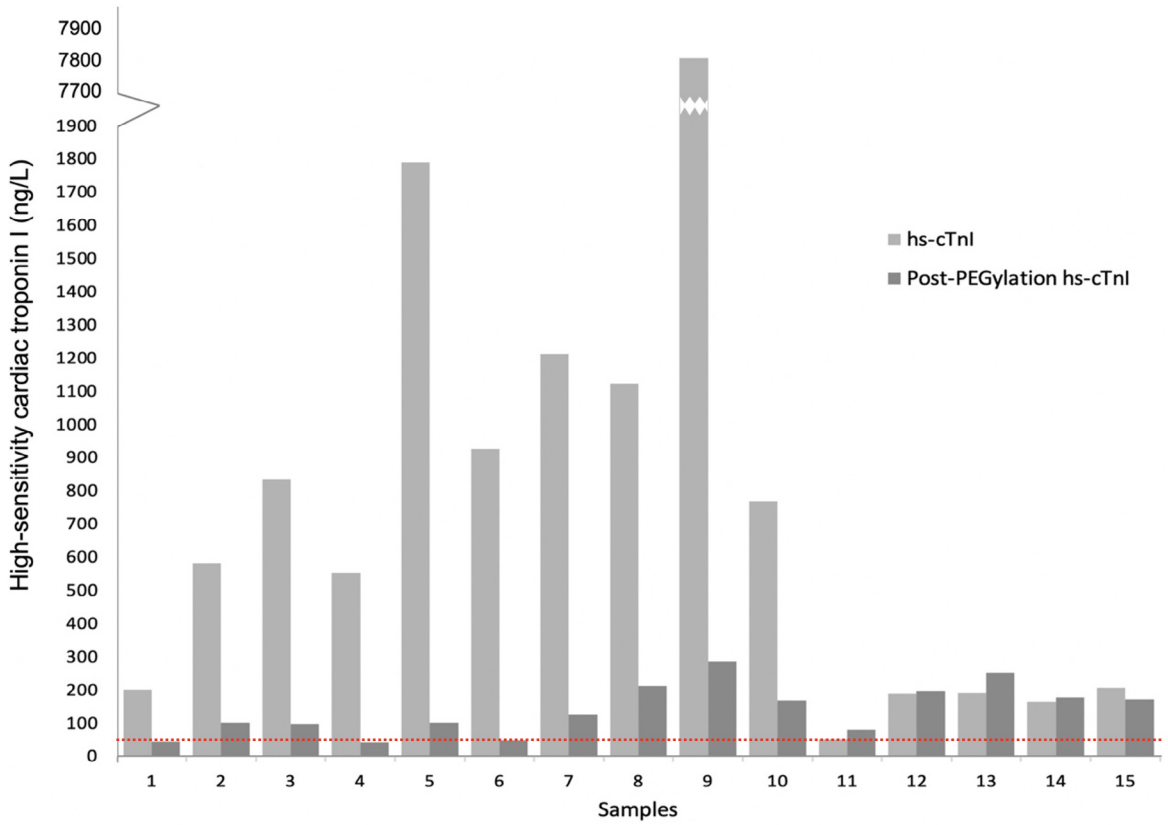

Figure 1. High-sensitivity cardiac troponin I (hs-cTnI) levels of 15 patients with elevated troponin on routine clinical samples, prior to (light grey) and after treatment with polyethylene glycol (PEG) (dark grey). Dotted line indicates the hs-cTnI 50 ng/L threshold, above which values were considered to be elevated. Post-PEGylation recoveries less than 25% were demonstrated in samples one to ten, indicating the presence of macrotroponin.

Table 1. Demographic, clinical and biochemical characteristics of ten patients in whom macrotroponin was identified.

Age	Sex	Genotype	Phenotype	Treatment (ERT)	eGFR (mL/min /1.73 m^2)	TnI (Atellica) (ng/L)	Post-HBT TnI (ng/L)	Post-PEG TnI (ng/L)	PEG recovery (%)
56	M	p.T141I c.422C>T Missense	Classical	Agalsidase beta	>90	7809	7554	286	3.7%
79	F	p.R301Q c.902G>A Missense	Variable, atypical	No treatment	85	1789†	2120	100	5.6%
60	F	p.W340* c.1019G>A Nonsense	Classical	No treatment	76	1211	1345	125	10.3%
55	M	p.Y365* c.1095T>A Nonsense	Classical	Agalsidase beta	40	1122	1179	211	18.8%
61	F	p.W340* c.1019G>A Nonsense	Classical	Agalsidase alpha	63	926	951	46	5.0%
63	F	p.L311Ffs*6 c.931delC Frameshift/deletion	Classical	Agalsidase alpha	>90	833	753	97	11.6%
64	F	p.G328E c.983G>A Missense	Classical	No treatment	>90	768	770	167	21.7%
51	F	p.C202W c.606T>G Missense	Classical	Agalsidase beta	85	580	572	101	17.4%
59	F	p.G373Pfs*1 c.1114_1115 insTCCC Frameshift/insertion	Classical	No treatment	>90	551	528	40	7.3%
51	F	p.Q330Sfs*18 c.988del Frameshift/deletion	Classical	Agalsidase beta	>90	200	204	42	21.0%

All TnI assays measured using Siemens Atellica High Sensitivity Troponin-I assay, except: †Abbott (N≤16 ng/L)
ERT = Enzyme Replacement Therapy; HBT = Heterophilic Blocking Tube; PEG = Polyethylene Glycol

Discussion

This case series presents the first of its kind, in describing the prevalence of macrotroponin in Fabry disease. We demonstrated that in a clinically stable cohort of 47 FD patients, 32% had elevated hs-cTnI, in the absence of acute coronary symptoms. Of these, macrotroponin was identified in two-thirds of patients with elevated hs-cTnI, as demonstrated by low post-PEGylation hs-cTnI recovery (< 25%), suggesting the presence of high molecular weight troponin I complexes. As a proportion, this is markedly higher than the prevalence of macrotroponin reported in non-Fabry patients [15]. The other one-third of patients with elevated hs-cTnI had true elevations of troponin, although these were substantially lower in magnitude than the macrotroponin group and predominantly occurred in males. There are multiple potential reasons underpinning these elevations in baseline troponin, including troponin leak or underlying conditions associated with FD, such as cardiomyopathy and renal impairment [1]. As such, this case series raises the dual questions of whether FD patients have a greater underlying predisposition to macrotroponin, and what factors underpin the true elevations in troponin that are observed in higher prevalence in these patients. Additionally, the female preponderance in macrotroponin demonstrated in this case series also raises the question of whether there is a possible underlying autoimmune association.

The potential diagnostic dilemma brought about by macrotroponin has prompted recent research into its prevalence and detection. To date, studies have not consistently agreed upon a prevalence of macrotroponin in the general population, due to the significant amount of inter-assay variability. A 2016 Australian study found that among approximately 3900 samples from patients presenting to emergency departments predominantly, approximately 1100 samples registered a hs-cTnI above the sex-specific 99th percentile, as per the Architect High Sensitive Troponin I and VITROS Troponin I ES assays [15]. Of this group, macrotroponin was identified in 5% of patients, as defined by post-PEGylation hs-cTnI recovery less than 15%.

Contrastingly, a 2020 New Zealand study found quite a high prevalence of macrotroponin, with 76% of their cohort of 223 community laboratory specimens demonstrating elevated cTnI, and 55% demonstrating macrotroponin [16]. Of note, this study defined macrotroponin as a cTnI recovery less than 40% using the Siemens hs-cTnI Centaur assay after protein A immunoglobulin depletion, and validated this with additional methods of gel filtration and PEG precipitation [16].

As it currently stands, there is no established consensus in the literature on the gold standard assay for detection of macrotroponin, nor the percentage recovery threshold that defines macrotroponin. It was proposed that the 55% recovery in the previously-described study was attributable to the larger threshold in the definition of macrotroponin, as well as the higher sensitivity of the Centaur assay [16]. The 40% threshold selected by the study was empirically determined, based on the bimodal distribution of recovery after protein A immunoglobulin depletion. However, the majority of cases of macrotroponin in the study registered recovery levels less than 20%. The distribution of findings in our population demonstrated the 25% post-PEGylation recovery threshold to be the natural cut-off point that clearly distinguished subjects into a macrotroponin and non-macrotroponin group, with lowest recovery percentage of 83% in the non-macrotroponin group.

This case series aimed to provide preliminary insight into the prevalence of macrotroponin in FD patients, however several limitations ought to be acknowledged. Owing to the case series nature of this work, we sampled a small cohort of 47 patients who were receiving routine clinical testing. As such, while these findings do provide a snapshot, further larger-scale studies are required to accurately quantify the prevalence of macrotroponin in FD. Given the lack of consensus in the literature as to the true prevalence of macrotroponin in the general population, it would be of benefit to extend this study by comparing troponin and macrotroponin in a larger study with both an FD and control group. Furthermore, our case series only sought to capture troponin concentrations at one timepoint in the patient's routine care. As such, a longitudinal understanding of the patient's troponin concentration and macrotroponin status has not been established. While it appears that macrotroponin was identified in our study among patients from varying ages, genotypes, phenotypic expressions, treatment statuses and renal function, associations between these factors and macrotroponin cannot be made in the absence of a formal statistical analyses with larger numbers and data.

This case series highlights the diagnostic dilemma that may emerge in clinical settings if macrotroponin is not recognised. This is especially relevant for the unique Fabry cohort in whom baseline cardiovascular risk and troponin are elevated [1], making these patients susceptible to misdiagnosis and a cascade of potentially unnecessary investigations and interventions. However, it is also possible that both true elevations in troponin and macrotroponin may occur simultaneously, which can lead to conflicting results and questionable hs-cTnI recoveries after PEG precipitation [15]. Compounding this, macrotroponin itself may pose a cardiotoxic effect that also contributes to the rise in cardiac troponin [17], despite it typically being considered non-pathogenic. Based on this case series, we recommend that clinicians consider the possibility of macrotroponin in patients with elevated cardiac troponin, particularly when biochemical results are discordant with the patient's clinical presentation.

In conclusion, this case series reports a relatively high prevalence of macrotroponin in FD patients with elevated high-sensitivity troponin I. Further larger-scale studies are required to accurately determine the prevalence of macrotroponin in FD patients, understand the factors that lead to elevated troponin and macrotroponin, and determine how this influences clinical management.

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Declaration of Conflicts of Interests

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