



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

## Antibody-mediated interferences affecting cardiac troponin assays

### Citation for published version:

Hammarsten, O, Warner, JV, Lam, L, Kavsak, P, Lindahl, B, Aakre, KM, Collinson, P, Jaffe, AS, Saenger, AK, Body, R, Mills, NL, Omland, T, Ordonez-Llanos, J & Apple, FS 2023, 'Antibody-mediated interferences affecting cardiac troponin assays: recommendations from the IFCC Committee on Clinical Applications of Cardiac Biomarkers', *Clinical Chemistry and Laboratory Medicine*. <https://doi.org/10.1515/cclm-2023-0028>

### Digital Object Identifier (DOI):

[10.1515/cclm-2023-0028](https://doi.org/10.1515/cclm-2023-0028)

### Link:

[Link to publication record in Edinburgh Research Explorer](#)

### Document Version:

Publisher's PDF, also known as Version of record

### Published In:

Clinical Chemistry and Laboratory Medicine

### General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

### Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



## Guidelines and Recommendations

Ola Hammarsten\*, Janet V. Warner, Leo Lam, Peter Kavsak, Bertil Lindahl, Kristin M. Aakre, Paul Collinson, Allan S. Jaffe, Amy K. Saenger, Richard Body, Nicholas L. Mills, Torbjørn Omland, Jordi Ordonez-Llanos and Fred S. Apple

# Antibody-mediated interferences affecting cardiac troponin assays: recommendations from the IFCC Committee on Clinical Applications of Cardiac Biomarkers

<https://doi.org/10.1515/cclm-2023-0028>

Received January 10, 2023; accepted January 12, 2023;

published online March 24, 2023

**Abstract:** The International Federation of Clinical Chemistry Committee on Clinical Applications of Cardiac Biomarkers (IFCC C-CB) provides educational documents to facilitate the interpretation and use of cardiac biomarkers in clinical laboratories and practice. Our aim is to improve the understanding of certain key analytical and clinical aspects of cardiac biomarkers and how these may interplay. Measurements of cardiac troponin (cTn) have a prominent place in the clinical work-up of patients with suspected acute coronary syndrome. It is therefore important that clinical

laboratories know how to recognize and assess analytical issues. Two emerging analytical issues resulting in falsely high cTn concentrations, often several fold higher than the upper reference limit (URL), are antibody-mediated assay interference due to long-lived cTn-antibody complexes, called macrotroponin, and crosslinking antibodies that are frequently referred to as heterophilic antibodies. We provide an overview of antibody-mediated cTn assay interference and provide recommendations on how to confirm the interference and interpret the results.

**Keywords:** assay interference; cardiac troponin; heterophile antibodies; immune complexes; macrotroponin; myocardial infarction.

\*Corresponding author: **Ola Hammarsten**, MD, PhD, Professor, Senior Physician, Institute of Biochemistry, Department of Clinical Chemistry and Transfusion Medicine, University of Gothenburg, Bruna stråket 16, Sahlgrenska Academy at the University of Gothenburg, 41345 Gothenburg, Sweden, Phone: +46 31 342 1000, Fax: +46 31 82 84 58, Mobile: +46 733 200834, E-mail: [ola.hammarsten@clinchem.gu.se](mailto:ola.hammarsten@clinchem.gu.se)

**Janet V. Warner**, Faculty of Medicine, The University of Queensland, Saint Lucia, Australia

**Leo Lam**, Chemical Pathology, LabPlus, Auckland City Hospital, Auckland, New Zealand; and Biochemistry, Middlemore Hospital Laboratories, Auckland, New Zealand

**Peter Kavsak**, Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

**Bertil Lindahl**, Department of Medical Sciences, Uppsala University and Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden

**Kristin M. Aakre**, Department of Medical Biochemistry and Pharmacology and Department of Heart Disease, Haukeland University Hospital, Bergen, Norway; and Department of Clinical Science, University of Bergen, Bergen, Norway. <https://orcid.org/0000-0002-7340-6736>

**Paul Collinson**, Departments of Clinical Blood Sciences and Cardiology, St George's University Hospitals NHS Foundation Trust, London, UK; and St

George's University of London, London, UK. <https://orcid.org/0000-0002-7000-5996>

**Allan S. Jaffe**, Departments of Laboratory Medicine and Pathology and Cardiology, Mayo Clinic, Rochester, MN, USA

**Amy K. Saenger and Fred S. Apple**, Department of Laboratory Medicine and Pathology, Hennepin Healthcare/HCMC, Minneapolis, MN, USA; and Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA

**Richard Body**, Emergency Department, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK; Division of Cardiovascular Sciences, The University of Manchester, Manchester, UK; and Healthcare Sciences Department, Manchester Metropolitan University, Manchester, UK

**Nicholas L. Mills**, BHF/University Centre for Cardiovascular Science and Usher Institute, University of Edinburgh, Edinburgh, UK

**Torbjørn Omland**, Department of Cardiology, Akershus University Hospital, Lørenskog, Norway; and Institute of Clinical Medicine, University of Oslo, Oslo, Norway

**Jordi Ordonez-Llanos**, Servicio de Bioquímica Clínica, Hospital de Sant Pau, Barcelona, Spain; and Foundation for the Biochemistry and Molecular Pathology, Barcelona, Spain

## Introduction

Cardiac troponin T (cTnT) and I (cTnI) are widely used biomarkers in the evaluation of patients with suspected acute coronary syndrome and are integral to the diagnosis of acute myocardial infarction (MI) [1]. The ability to measure very low cTn concentrations using high-sensitivity assays has improved diagnostic accuracy, allowed for more rapid triage of patients without increased admissions, and in some instances reduced hospital costs [2, 3]. However, there are many instances where the etiology of an increased cTn remains unclear. Some of these alternative etiologies are tabulated in Table 1. Clinically, chronic cTn increases portend a poor prognosis as underlying structural or coronary heart disease are associated with a higher risk of future cardiovascular events, especially when due to non-ischemic causes [4, 5], and one needs a diagnosis to determine optimal management. On the other hand, false cTn elevations may result in further examinations, including cardiac imaging and coronary angiography [6], that can be associated with some risk to the patient and unnecessary hospital costs [7, 8].

**Table 1:** Conditions associated with cTn elevations.

<b>Cardiac causes</b>
Myocardial infarction
Coronary revascularization procedure
Heart failure
Myocarditis
Cardiomyopathy
Takotsubo syndrome
Cardiac procedure other than revascularization
Catheter ablation
Defibrillator shocks
Cardiac contusion
Sustained tachyarrhythmia
Severe hypertension
<b>General conditions affecting myocardium</b>
Sepsis, infectious disease
Chronic kidney disease
Infiltrative diseases, e.g., amyloidosis, sarcoidosis
Chemotherapeutic agents
Critically ill patients
Strenuous exercise
Hypertrophy
Respiratory failure
Severe anaemia
Hypotension or shock
Stroke, subarachnoid haemorrhage
Pulmonary embolism, pulmonary hypertension

Modified from [1].

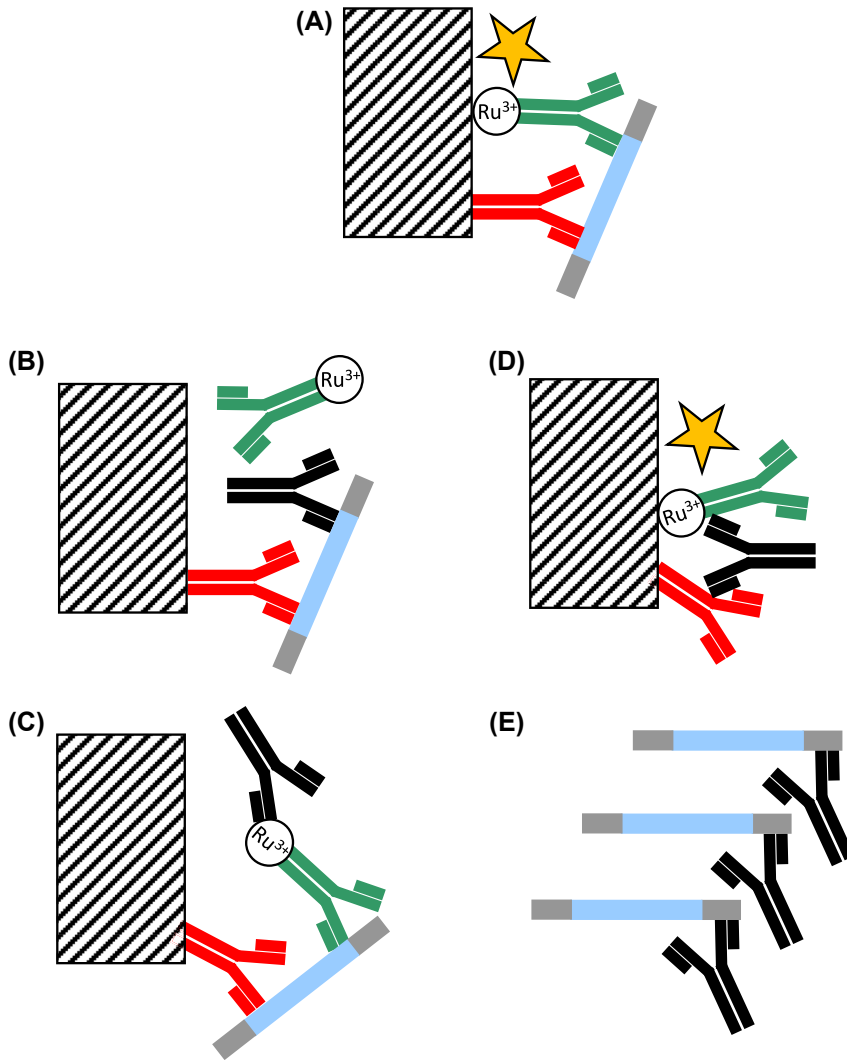
This was underlined by a recent study of patients admitted to hospital that indicated that those with evidence of cTn macrotroponin had lower mortality and a lower frequency of acute cardiac disease compared to patients with increased cTn but no evidence of interference [9].

All immunoassays, including those used to measure cTn are vulnerable to interferences that can result in erroneous measurement and thus imperfect clinical decisions. The ability to find analytical errors and interferences [10–12], and to establish a laboratory protocol to identify interferences if a clinician questions a cTn result, is the responsibility of the clinical laboratory [13]. The collected experience is that the vast majority of stable cTn increases that prompt referral to laboratories are due to a cTn-assay interference, suggesting that a clinical suspicion of interference can be quite specific. However, this may in part be due to limited awareness of cTn-assay interference amongst clinicians and under recognition in practice. The literature on immunoassay interference is complex but our collected experience is that interferences that result in stable falsely increased cTn concentrations are primarily mediated by IgG antibodies. This limits the type of evaluation protocols that we recommend should be available in the central laboratory to investigate stable cTn elevations that are questioned by the clinician. cTn interferences resulting in falsely low concentrations or by non-antibody mediated mechanisms are seldom identified clinically, although they do exist [14, 15].

The literature on troponin interference is limited and no guidance exists either for clinicians or laboratories on how to handle this problem, often leading to repetitive investigations and potential complications for the affected patients. The purpose of this article is to give an overview of the current literature and, based on that and our collective experience, provide guidance for clinical laboratories on how to identify interferences affecting cTnI and cTnT assays.

## Mechanisms involved in cTn-assay interference

The most common cTn-assay anomalies are outliers, also called “fliers”, when the results between two successive analyses of cTn of the same sample vary significantly sometimes due to fibrin-clots [8]. However, the most common reason for false stable increases of cTn that prompt clinical suspicion of interference are mediated by patient IgG antibodies [16, 17]. It is therefore particularly important that the central laboratory, in addition to dealing with fliers, can identify and interpret IgG-mediated false cTn increases in a reliable and safe way.



**Figure 1:** Possible mechanisms behind antibody-mediated cTn assay interference. (A) Typical immunoassay with one capture (red) and one Ruthenium labeled detection assay antibody (green) that colocalize on a cTn molecule (gray and blue bar) and generate a signal when the Ruthenium ion is brought in close proximity to an electrode. (B, C) Blocking anti-cTn antibodies. If patient anti-cTn antibodies (black) block assay antibodies from binding to cTn or generating a signal, the cTn assay may generate false negative results. (D) Heterophilic antibodies. Patient antibodies that crosslink assay antibodies can generate cTn-independent signals and result in false cTn-elevations. (E) Macro-troponin. Patient anti-cTn antibodies may form long lived antibody-cTn complexes, and result in a build-up of stable cTn increases that may not indicate an increased cTn release from the heart. (Figure based on the electrochemiluminiscent cTnT assay from Roche Diagnostics).

cTn immunoassays have two or more antibodies that must bind to the same cTn molecule to generate a measurable signal (Figure 1A). The cTn concentration in a patient's sample will be underestimated if anti-cTn antibodies are present in the patient's blood and compete with the assay antibodies for binding to cTn, which results in blocking the signal generation (Figure 1B and C). Conversely, cTn concentrations will be overestimated if patient antibodies crosslink the cTn-assay antibodies independently of cTn binding (Figure 1D). These are referred to as heterophilic antibodies and include human anti-mouse antibodies (HAMA) and rheumatoid factors. A third type of interference is caused by the formation of immunocomplexes between patient anti-cTn antibodies and cTn, as shown in Figure 1E, and is known as macro-troponin. Immunoglobulins have a half-life over several weeks, whereas free cTn has a half-life of a few hours [18, 19]. Therefore, since the healthy heart constantly releases some cTn, circulating macro-troponin complexes persist and result in a higher-than-normal steady state level of cTn that may not indicate a stable increased cTn

release from the heart due to injury. Consistent with this assumption, some studies have reported a lower risk of death and cardiac events among patients with cTn increases due to macro-troponin [9, 20].

As with most immunoassays, interference is often assay-dependent, likely because heterophile antibodies can be quite specific and may or may not bind to the cTn-assay antibodies (Figure 1D). Similarly, circulating cTn degradation products may or may not be involved in the macro-troponin complex or be measured by a given cTn-assay.

## The frequency of cTn-assay interference

Different studies have reported very different prevalence of cTn-assay interference possibly because estimates are not only dependent on the cTn measured, but also on the cTn-assay design and population [17, 21]. When detected by

discordant results from two cTnI assays on 3,897 individuals and then further analyzed by removal of IgG by protein A, blocking of heterophile antibodies or gel filtration chromatography, the prevalence was 5% of patients with cTnI concentrations above sex-specific 99th percentile URLs [16]. Discordance for cTnI assays between one contemporary sensitive cTnI and a high-sensitive cTnI assay in 2,658 patients was 1.2% [22]. In one study interference was investigated in all samples received from primary care that showed cTnI concentrations above the URL for a cTnI assay. Evidence of interference was shown for 123/223 samples when analyzed before and after removal of IgG by protein A resin [17]. Of the 123 samples with evidence of cTn-assay interference in the first cTn-assay only 10 samples had evidence of interference in all of the six cTn-assay included in the study showing that cTn-assay interference is often assay specific [9]. Finally, cTnI assay interference was 17% of the analyses when 9 different assay platforms were tested on samples from 10 patients with autoimmune disease. In this study, cTnI was one of 5 analytes most commonly affected by assay interference among the 74 analytes included in the study [23]. Finally, the prevalence of cTnI-assay interference appears higher than for cTnT-assay interference [17].

In conclusion, although the true frequency of cTn assay interference is not known it likely constitutes several percent of all elevated cTn results on some cTn assay platforms.

## Indications for cTn assay interference analysis

Interference testing and information about this problem with cTn-assays should be provided by the central laboratory. Current methods for identification of cTn assay interference are manual, labor intensive and time-consuming, potentially delaying necessary treatment of acute cardiac conditions. Interference testing should only be undertaken in consultation between laboratory and clinician if cTn assay interference is suspected, after other analytical causes such as fibrin-clots and pre-analytical factors such as hemolysis are ruled-out and where interpretative expertise and clinical information are available.

Scenarios in which cTn assay interference are likely to be present are listed in Table 2.

Recommendation #1: cTn-assay interference analysis should be done when there is a reasonable clinical suspicion that a cTn result is possibly incorrect or incongruent with the clinical findings.

**Table 2:** Findings indicating that cTn-assay interference may cause cTn elevation.

---

### Non-acute conditions

---

Stable cTn elevation in the absence of a clear reason after clinical work-up. cTn elevation to very different levels using different cTn assays in patients without acute myocardial infarction.

cTn levels show an unreasonable variation over time, after reanalysis of the same sample, after reanalysis in another sample type (plasma/serum) or after dilution of the patient sample.

---

### Acute conditions

---

cTn elevation higher than expected during an acute cardiac event. cTn levels linger on after an acute cardiac event.

---

Recommendation #2: Laboratories should provide a service for cTn-assay interference investigations.

## Methods for detecting cTn assay interference

If there is clinical suspicion of cTn-assay interference we recommend repeat testing with the initial cTn assay utilized, following re-centrifugation, dilution or with an alternate specimen type (serum or plasma) [24–26]. If discrepant results persist the first-in-line method should be to retest specimen using a different cTn assay. If results are unclear other methods, like PEG precipitation, listed in Table 3 and in the flowchart (Figure 2) can be used if available. These methods are described in more detail below.

## Repeat analysis with a different cTn-assay

Discordant results between different hs-cTn assays can be a sign of potential cTn assay analytical interference [17, 21].

Assay interference may be inferred if the clinical suspicion of cardiac disease is low, the increase in cTn remains stable over time, and either cTn concentrations are below the URL with an alternative assay or if cTn concentrations between two assays differ by more than 3–5 fold in conditions other than acute myocardial infarction [16, 22]. Our experience is that discordant troponin results between different assays are seen in approximately 50% of cases where analytical interferences are later confirmed. Some medical centers have implemented two different cTn assays to assess for the presence of analytical interferences

**Table 3:** Methods used in investigations of possible cTn-assay interference.

Routine lab methods				
Method	Mode of action	Analysis time	Pros	Cons
Alternative hs-cTn assay	Variable	<1 h	Easy. Fast. Inexpensive. Extensively used in clinical routine.	May miss cTn-assay interferences.
Polyethylene glycol (PEG) precipitation	Precipitation of large molecules including immunoglobulins	1 h	Easy. Fast. Inexpensive. Extensively used in clinical routine.	Exactly what is precipitated in patient sample not known. Different cTn assays are affected differently by presence of PEG in the sample.
Dilution	Unknown	<1 h	Easy. Fast. Inexpensive. Extensively used in clinical routine.	May miss cTn-assay interferences.
Reference lab methods				
Protein A/G spin column	Removal of IgG	1 h	Specific removal of IgG. Easy. Fast. Inexpensive.	Only IgG mediated interference will be detected. Not available in most labs.
Heterophile blocking reagent	Blocking of crosslinking antibodies	<1 h	Easy. Fast. Inexpensive. Extensively used in clinical routine.	Do not detect macrotroponin. Heterophile antibodies may be an uncommon cause of interference.
Gel filtration chromatography	Separation based on molecular weight	24 h	Will find any type of antibody mediated interference.	Slow and labor intensive. One sample at a time. Requires high laboratory skills. Requires special equipment such as chromatography equipment.
Sucrose gradient ultracentrifugation	Separation based on molecular weight	24 h	Will find any type of antibody mediated interference. Many samples can be run simultaneously.	Slow and labor intensive. Not an established technique in most labs. Requires high laboratory skills. Requires special equipment such as fluorometer and ultracentrifuge.

[16, 22, 27]. Furthermore, the laboratory may also collaborate with other local laboratories which utilize different cTn assays to aid in investigating potential interferences and possibly finding a cTn-assay without interference on individual patients.

On rare occasions, the interferent can involve both assays which may cause confusion. Importantly, truly discordant cTnI and cTnT concentrations may be present in acute MI when cTnI concentrations are often greater than cTnT during the first days [28]. In patients with chronic neuromuscular skeletal disease, cTnT assays have demonstrated real cTnT release from diseased skeletal muscle in approximately 50% of patients not representing myocardial injury, while cTnI assays are 100% myocardial tissue specific [29, 30].

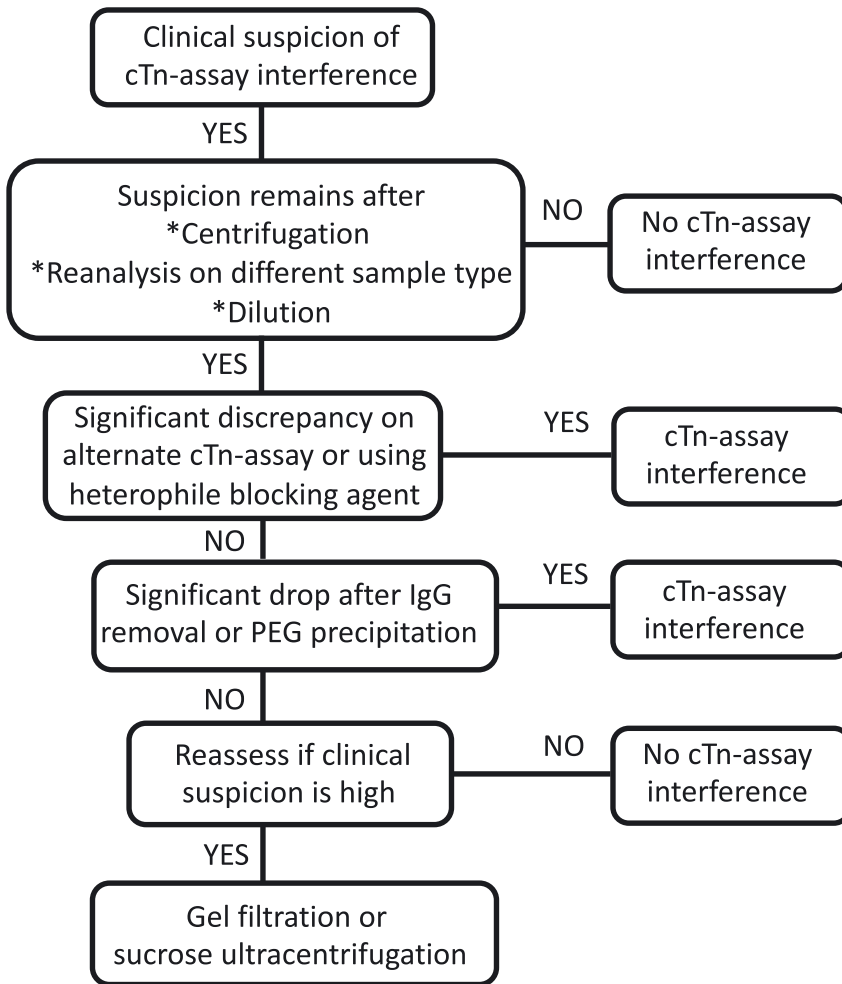
## Polyethylene glycol precipitation

An alternative method for the detection of cTn assay interferences is polyethylene glycol (PEG) precipitation using a protocol that precipitates most plasma proteins, including immunoglobulins [21]. The cTn concentration is measured before and after PEG precipitation. If the decrease in cTn-concentration is more than 80% (recovery <20%) or if

the decrease is significantly different from routine control samples, the stable cTn increase is likely due to patient antibody mediated cTn assay interference. This method is simple to perform, and most laboratories have experience with PEG precipitation procedures, as it is also used to investigate macroprolactin. There are published detailed protocols for interpretation in the context of other information such as serial dilutions and patient clinical data [31]. However, interpretation of results may still be convoluted [32]. PEG precipitation protocols promote non-specific precipitation of most proteins in the sample rather than those of a target single-antibody immune complex, thus PEG precipitation does not assess the origin of the patient's cTn assay interference. In addition, different cTn assays are affected by the presence of PEG in the sample to varying degrees, which may confound interpretation [31].

## Blocking agents for heterophilic antibodies

Patient specimens can be treated with commercially available heterophilic antibodies blocking reagent (e.g., HBR, Scantibodies Laboratory Inc., Santee, CA).



**Figure 2:** Suggested algorithm for investigation of suspected cTn-assay interference.

A significant decrease in the cTn concentration indicates a falsely increased cTn due to the presence of crosslinking antibodies. While the definition of a significant decrease is presently not clearly defined, among 24 case reports the median decrease in cTn concentration was 96% (IQR 73–97%) following HBR treatment when interference were present [33]. Studies that have systematically evaluated the effect of HBR treatment demonstrate that only a subset of suspected cTn-interferences due to discordant results between assays were identified with this method [34]. HBR is not formulated to react with macrotroponin, therefore this finding is not unexpected. As the composition of HBR is proprietary information, any interference detected by this method cannot be definitively explained.

## Immunoglobulin removal

The vast majority of falsely increased cTn concentrations reported in the literature are due to interferences from

circulating IgG. The most direct way to investigate a potential false cTn increase is to analyze cTn concentrations before and after removal of IgG by protein A [16, 17] or protein G [27] resins. Currently there are no automated methods for IgG removal, so spin columns with protein A and/or G resins, or beads alone are often used, sometimes as a kit [6]. Concurrent analysis of TSH [35] or ferritin [27] may be used to compensate for non-specific losses but that assessment is not always performed [16]. Complete removal of IgG can be determined but overloading the protein A or protein G spin columns is not a problem using published protocols [17, 27].

Patient antibody mediated cTn assay interference may be present if the cTn concentrations decrease more than 60% after removal of IgG (<40% recovery) [17] or if the decrease in cTn concentration is significantly different from routine control samples with true cTn elevations. Often, the decrease in cTn concentration is over 5-fold when the patient cTn increase is due to antibody interference and the interpretation is straight-forward as reported in around 70% of the

cases [27]. However, the within-sample variability in recovery of the protein G spin column method is over 10% which may add confusion [27]. Finally, rheumatoid factors are crosslinking IgM antibodies, according to most rheumatoid factor assays [36], and may be missed by the protein A/G method that mainly remove IgG. The extent of this potential problem is unclear [34, 37, 38] but caution should be used when interpreting results from the protein A/G method on patients with high rheumatoid factor titers. In these cases PEG precipitation, anti-IgM or anti-IgA resins may be used as a complementary method.

## Molecular weight methods

Most circulating cTn are degradation products with a molecular weight below 40 kDa [27], whereas the antibody-cTn complexes that cause macrotroponin and interfering antibodies have a molecular weight around 150 kDa. It is therefore possible to detect all types of patient antibody mediated cTn assay interference by methods that determine the native molecular weight of the measured cTn and could be viewed as reference methods. Methods that have been used in clinical practice to determine the molecular weight of cTn in circulation include gel filtration chromatography [16, 17, 39] and sucrose gradient ultracentrifugation [27]. Native gel electrophoresis can also separate proteins by weight [40].

Methods that determine native molecular weights are laborious and should not be the first-line method. However, sometimes results remain inconclusive after removal of immunoglobulins by protein A/G resins or PEG precipitation. In these situations, additional analysis of the native molecular weight of the measured cTn often aids in the interpretation and at our sites the results from molecular weight methods are always conclusive.

If available, specialized laboratories with gel filtration chromatography or sucrose gradient ultracentrifugation experience could be consulted to analyze cTn-interference in this manner, providing a valuable resource if PEG and/or protein A/G findings are inconclusive. Macro-troponin specimens are stable for over a week at +4 °C [27] and can be stored frozen for extended periods of time [35], allowing for this analysis by reference laboratories.

## Reporting of cTn assay interference

It is important in the post-analytical phase that residual cTn concentrations after removal of IgG or PEG precipitation are not automatically regarded as the patient's baseline cTn levels. Results from a cTn assay interference analysis should be

interpreted in the context of the individual patient and not simply reported as a before-and-after result in the patient's health record without comment or interpretation. Consultation with a laboratory professional is essential to explain these findings to the clinician and ensure that the finding is permanently recorded and easily accessible in the patient health record so that the clinicians will be alerted that future cTn results from this patient may be unreliable. The lab may also suggest how this patient's samples may be analyzed in the future, perhaps with an alternate cTn-assay.

**Recommendation #3:** The residual cTn concentrations after removal of IgG or after PEG precipitation should not be reported as the patient's cTn concentration in their health-care record.

**Recommendation #4:** cTn-assay interference analysis should be accompanied with an interpretation of the results and generate a permanent, easily accessible entry in the patient's healthcare record.

**Research funding:** OH is supported by The Swedish Cancer Society, the Swedish Heart and Lung Foundation and LUA/ALF funding at the Sahlgrenska University Hospital. NLM is supported by a Chair Award, Programme Grant, and Research Excellence Award (CH/F/21/90010, RG/20/10/34966, RE/18/5/34216) from the British Heart Foundation.

**Author contributions:** All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

**Conflicts of interest:** OH has received honoraria from Siemens Healthineers and LumiraDx and has stock and stock options from AlignedBio. Grants: The Swedish Cancer Society, the Swedish Heart and Lung Foundation and LUA/ALF funding at the Sahlgrenska University Hospital. NLM has received research grants or honoraria from Abbott Diagnostics, Roche Diagnostics, Siemens Healthineers and LumiraDx. Grants: supported by a Chair Award, Programme Grant, and Research Excellence Award (CH/F/21/90010, RG/20/10/34966, RE/18/5/34216) from the British Heart Foundation FSA: Consultant: HyTest Ltd, AWE Medical Group; Associate Editor: Clinical Chemistry; Advisory Boards: Werfen, Siemens Healthineers, Qorvo, Abbott Vascular; Honorarium for Speaking at Industry Conferences: Siemens Healthineers, Beckman Coulter; PI on Industry Funded Grants (non-salaried) on cardiac biomarkers through Hennepin Healthcare Research Institute: Abbott Diagnostics, Abbott POC, BD, Beckman Coulter, Ortho-Clinical Diagnostics, Roche Diagnostics, Siemens Healthcare, ET Healthcare, Qorvo. PO has received honoraria from Siemens Healthineers; Advisory board: Radiometer, Psyros diagnostics, Siemens Healthineers, LumiraDx. Associate Editor Journal of Applied Laboratory Medicine. BL, LM, JW reports no



relevant conflict of interest. JO-LI reports: Consultant fees from AWE Medical and Hemcheck; Advisory boards: Siemens Healthineers. TO reports grants from Novartis, Abbott Diagnostics, Roche Diagnostics, honoraria from CardiNor, Bayer, Abbott, has a pending patent application for GDF-15 for Predicting the Disease Severity of a Patient With COVID-19, and has stock and stock options from CardiNor. PK has received grants from Abbott Laboratories, Beckman Coulter, Ortho Clinical Diagnostics, Randox Laboratories, Roche Diagnostics, Siemens Healthcare Diagnostics; Consulting fees from Abbott Point of Care, Beckman Coulter, Roche Diagnostics, Quidel, Siemens Healthcare Diagnostics; Honoraria: Beckman Coulter, Roche Diagnostics, Siemens Healthcare Diagnostics, Thermo Fisher Scientific; Patents: McMaster University has also filed patents with Dr. Kavsak listed as an inventor on Quality Control Materials for Cardiac Troponin Testing and Identifying pregnant women at increased risk for hypertension and future cardiovascular disease McMaster University has filed a patent with Dr. Kavsak listed as an inventor in the acute cardiovascular biomarker field, in particular, a patent has been awarded in Europe (EP 3 341 723 B1) on a Method of determining risk of an adverse cardiac event. PB has received grants from National Institute for Health Research, British Lung Foundation, Asthma UK, Department of Health and Social Care, Siemens Healthineers, Abbott Point of Care, Ancon; consulting for Roche, Siemens, Psyros Diagnostics, Aptamer Group, LumiraDx, Beckman Coulter. Radiometer; Honoraria: EMCREG International; Support for attending meeting: Roche, EMCREG International Advisory board: FORCE Trial, REWIRE Trial, TARGET-CTA. Equimplent and materials from Roche, LumiraDx, Chronomics, My110, BD, iXensor, Abbott Point of Care, Randox Laboratories, Avacta, Menarini, Ancon; Leadership or fiduciary role: Deputy National Specialty Lead for Trauma & Emergency Care, National Institute for Health and Care Research Clinical Research Network, Magnetocardiography study (MAGNETIC) sponsored by Creavo. AKS advisory board: Radiometer, LumiraDx. AJ has received consulting fees from Abbott, Radiometer, Siemens, LumiraDx, Roche, Astellas, Ortho Diagnostics, Beckman-Coulter, ET Healthcare, Sphingotec, Spinchip, Amgen, Novartis. Stock options: RCE Technologies.

**Informed consent:** Not applicable.

**Ethical approval:** Not applicable.

## References

1. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction (2018). *Eur Heart J* 2019;40:237–69.

2. Lopez-Ayala P, Boeddinghaus J, Koehlin L, Nestelberger T, Mueller C. Early rule-out strategies in the emergency department utilizing high-sensitivity cardiac troponin assays. *Clin Chem* 2021;67:114–23.
3. Bjurman C, Zywczyk M, Lindahl B, Carlsson T, Johanson P, Petzold M, et al. Decreased admissions and hospital costs with a neutral effect on mortality following lowering of the troponin T cutoff point to the 99th percentile. *Cardiol J* 2017;24:612–22.
4. Roos A, Bandstein N, Lundback M, Hammarsten O, Ljung R, Holzmann MJ. Stable high-sensitivity cardiac troponin T levels and outcomes in patients with chest pain. *J Am Coll Cardiol* 2017;70:2226–36.
5. Horiuchi Y, Wettersten N, Patel MP, Mueller C, Neath SX, Christenson RH, et al. Prognosis is worse with elevated cardiac troponin in nonacute coronary syndrome compared with acute coronary syndrome. *Coron Artery Dis* 2022;33:376–84.
6. Bionda C, Rousson R, Collin-Chavagnac D, Manchon M, Chikh K, Charrie A. Unnecessary coronary angiography due to false positive troponin I results in a 51-year-old man. *Clin Chim Acta* 2007;378:225–6.
7. Warner JV, Lam L. Macro-troponin probably contributes to a difference in patient stratification in suspected acute coronary syndromes. *J Am Coll Cardiol* 2021;78:295–6.
8. Nevraumont A, Deltombe M, Favresse J, Guillaume L, Chapelle V, Twerenbold R, et al. Interferences with cardiac biomarker assays: understanding the clinical impact. *Eur Heart J* 2022;43:2286–8.
9. Lam L, Tse R, Gladding P, Kyle C. Effect of macro-troponin in a cohort of community patients with elevated cardiac troponin. *Clin Chem* 2022; 68:1261–71.
10. Kittanakom S, Ly V, Arnoldo A, Beattie A, Kavsak PA. Pre-analytical variables affecting discordant results on repeat sample testing for cardiac troponin I. *Clin Biochem* 2019;63:158–60.
11. Kavsak PA, Ainsworth C, Worster A. An approach to investigating discordant high-sensitivity cardiac troponin I results. *Can J Cardiol* 2021; 37:1292–3.
12. Favresse J, Bayart JL, Gruson D, Bernardini S, Clerico A, Perrone M. The underestimated issue of non-reproducible cardiac troponin I and T results: case series and systematic review of the literature. *Clin Chem Lab Med* 2021;59:1201–11.
13. Favresse J, Burlacu MC, Maiter D, Gruson D. Interferences with thyroid function immunoassays: clinical implications and detection algorithm. *Endocr Rev* 2018;39:830–50.
14. Eriksson S, Junikka M, Laitinen P, Majamaa-Voltti K, Alfthan H, Pettersson K. Negative interference in cardiac troponin I immunoassays from a frequently occurring serum and plasma component. *Clin Chem* 2003;49:1095–104.
15. Bohner J, von Pape KW, Hannes W, Stegmann T. False-negative immunoassay results for cardiac troponin I probably due to circulating troponin I autoantibodies. *Clin Chem* 1996;42:2046.
16. Warner JV, Marshall GA. High incidence of macro-troponin I with a high-sensitivity troponin I assay. *Clin Chem Lab Med* 2016;54:1821–9.
17. Lam L, Aspin L, Heron RC, Ha L, Kyle C. Discrepancy between cardiac troponin assays due to endogenous antibodies. *Clin Chem* 2020;66: 445–54.
18. Starnberg K, Friden V, Muslimovic A, Ricksten SE, Nystrom S, Forsgard N, et al. A possible mechanism behind faster clearance and higher peak concentrations of cardiac troponin I compared with troponin T in acute myocardial infarction. *Clin Chem* 2020;66:333–41.
19. Friden V, Starnberg K, Muslimovic A, Ricksten SE, Bjurman C, Forsgard N, et al. Clearance of cardiac troponin T with and without kidney function. *Clin Biochem* 2017;50:468–74.

20. Lam L, Ha L, Gladding P, Tse R, Kyle C. Effect of macrotroponin on the utility of cardiac troponin I as a prognostic biomarker for long term total and cardiovascular disease mortality. *Pathology* 2021;53:860–6.
21. Kavsak PA, Roy C, Malinowski P, Mark CT, Scott T, Clark L, et al. Macrocomplexes and discordant high-sensitivity cardiac troponin concentrations. *Ann Clin Biochem* 2018;55:500–4.
22. Hasselbalch RB, Kristensen JH, Jorgensen N, Strandkjaer N, Alaour B, Afzal S, et al. High incidence of discrepancies in new Siemens assay - a comparison of cardiac troponin I assays. *Clin Chem Lab Med* 2022;60:921–9.
23. Marks V. False-positive immunoassay results: a multicenter survey of erroneous immunoassay results from assays of 74 analytes in 10 donors from 66 laboratories in seven countries. *Clin Chem* 2002;48:2008–16.
24. Mair J, Giannitsis E, Mills NL, Mueller C. Study Group on Biomarkers of the European Society of Cardiology Association for Acute CardioVascular C. How to deal with unexpected cardiac troponin results. *Eur Heart J Acute Cardiovasc Care* 2022;11:e1–3.
25. Lakusic N, Merkas IS, Lucinger D, Mahovic D. Heterophile antibodies, false-positive troponin, and acute coronary syndrome: a case report indicating a pitfall in clinical practice. *Eur Heart J Case Rep* 2021;5:ytab018.
26. Marinheiro R, Amador P, Parreira L, Rato Q, Caria R. False positive troponin I rendering two admissions for “recurrent acute myopericarditis”. *Open Cardiovasc Med J* 2018;12:55–8.
27. Hammarsten O, Becker C, Engberg A. Methods for analyzing positive cardiac troponin assay interference. *Clin Biochem* 2023;116:24–30.
28. Solecki K, Dupuy AM, Kuster N, Leclercq F, Gervasoni R, Macia JC, et al. Kinetics of high-sensitivity cardiac troponin T or troponin I compared to creatine kinase in patients with revascularized acute myocardial infarction. *Clin Chem Lab Med* 2015;53:707–14.
29. Rittoo D, Jones A, Lecky B, Neithercut D. Elevation of cardiac troponin T, but not cardiac troponin I, in patients with neuromuscular diseases: implications for the diagnosis of myocardial infarction. *J Am Coll Cardiol* 2014;63:2411–20.
30. du Fay de Lavallaz J, Prepoudis A, Wendebourg MJ, Kesenheimer E, Kyburz D, Daikeler T, et al. Skeletal muscle disorders: a noncardiac source of cardiac troponin T. *Circulation* 2022;145:1764–79.
31. Lafreniere MA, Tandon V, Ainsworth C, Nouri, Mondoux SE, Worster A, et al. Storage conditions, sample integrity, interferences, and a decision tool for investigating unusual high-sensitivity cardiac troponin results. *Clin Biochem* 2022;S0009-9120(22)00147-3.
32. Veljkovic K, Servedio D, Don-Wauchope AC. Reporting of post-polyethylene glycol prolactin: precipitation by polyethylene glycol 6000 or polyethylene glycol 8000 will change reference intervals for monomeric prolactin. *Ann Clin Biochem* 2012;49:402–4.
33. Lippi G, Aloe R, Meschi T, Borghi L, Cervellin G. Interference from heterophilic antibodies in troponin testing. Case report and systematic review of the literature. *Clin Chim Acta* 2013;426:79–84.
34. Onuska KD, Hill SA. Effect of rheumatoid factor on cardiac troponin I measurement using two commercial measurement systems. *Clin Chem* 2000;46:307–8.
35. Kavsak PA, Clark L, Caruso N, Bamford K, Lamers S, Hill S, et al. Macrocomplexes and high-sensitivity cardiac troponin assays in samples stored for over 15 years. *Clin Chim Acta* 2020;505:6–8.
36. Taylor P, Gartemann J, Hsieh J, Creeden J. A systematic review of serum biomarkers anti-cyclic citrullinated peptide and rheumatoid factor as tests for rheumatoid arthritis. *Autoimmune Dis* 2011;2011:815038.
37. Dasgupta A, Banerjee SK, Datta P. False-positive troponin I in the MEIA due to the presence of rheumatoid factors in serum. Elimination of this interference by using a polyclonal antisera against rheumatoid factors. *Am J Clin Pathol* 1999;112:753–6.
38. Kenny PR, Finger DR. Falsely elevated cardiac troponin-I in patients with seropositive rheumatoid arthritis. *J Rheumatol* 2005;32:1258–61.
39. Lam L, Ha L, Heron C, Chiu W, Kyle C. Identification of macrotroponin T: findings from a case report and non-reproducible troponin T results. *Clin Chem Lab Med* 2021;59:1972–80.
40. Akhtar Z, Dargan J, Gaze D, Firoozi S, Collinson P, Shanmugam N. False-positive troponin elevation due to an immunoglobulin-G-cardiac troponin T complex: a case report. *Eur Heart J Case Rep* 2020;4:1–5.