

CASE REPORT

False-Positive hs-cTnI in a Patient on Immunotherapy: a Case of Macromolecular Interference

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ABSTRACT

Background: High-sensitivity cardiac troponin (hs-cTn) is crucial for diagnosing acute coronary syndrome but is susceptible to analytical interference, leading to false-positive elevations (FPE). Macromolecular interferents (e.g., heterophilic antibodies or macrocomplexes) are known causes, especially in immunocompromised patients, such as those receiving immunotherapy [1,2].

Methods: A 62-year-old male with renal cell carcinoma on toripalimab presented with persistently elevated hs-cTnI levels without clinical signs of myocardial injury. Interference was assessed via polyethylene glycol (PEG) precipitation (using 25% PEG 6000), serial dilution, and alternative platform testing. PEG recovery < 20% indicated macromolecular interference.

Results: Initial hs-cTnI was 84.4 ng/mL (99th percentile URL = 24.2 ng/mL). Post-PEG, levels fell to 6.7 ng/mL (15.9% recovery), confirming interference. Serial dilution showed non-linearity. An alternate platform returned normal values. Subsequent hs-cTnI results remained normal, supporting transient interference.

Conclusions: Unexplained hs-cTnI elevations in immunotherapy-treated cancer patients should raise suspicion for macromolecular interference. PEG precipitation, dilution tests, and platform comparisons are effective verification tools. Clinician-laboratory collaboration is key to preventing misdiagnosis.

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KEYWORDS

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INTRODUCTION

High-sensitivity cardiac troponin (hs-cTn) testing has become the cornerstone for diagnosing acute coronary syndromes (ACS) due to its exceptional sensitivity in detecting myocardial injury [3]. However, this high sensitivity comes with a challenge: an increased vulnerability to analytical interference, which can lead to false-positive elevations (FPE) [4]. Such interferences may stem from heterophilic antibodies, autoantibodies, or macromolecular complexes like macrotroponin. These substances can bridge capture and detection antibodies

in sandwich immunoassays, generating false signals [2, 5].

The clinical implications of FPE are significant, potentially triggering unnecessary and invasive cardiac investigations, causing patient anxiety, and leading to inappropriate management [6]. This risk is particularly heightened in patients with cancer, especially those undergoing immunotherapy with agents such as immune checkpoint inhibitors (ICIs) [1]. The altered immune state in these patients may predispose them to the development of interferents. For instance, ICIs can potentially induce autoantibody production or form macrocomplexes that interfere with troponin assays [7]. While ICI-related myocarditis is a recognized, serious adverse effects distinguishing true myocardial injury from analytical interference are critical to avoid misdiagnosis [1, 8].

This report presents a case of a patient with renal cell carcinoma receiving toripalimab (an anti-PD-1 antibody) who exhibited persistent, asymptomatic hs-cTnI elevation. We detail the systematic laboratory investigation that identified and confirmed macromolecular interference using polyethylene glycol (PEG) precipitation, serial dilution, and platform comparison. This case underscores the necessity for vigilance and validation when interpreting abnormal troponin results in patients receiving immunotherapy.

CASE PRESENTATION

A 62-year-old male was admitted to the hospital with a chief complaint of episodic abdominal pain lasting for more than a week. Imaging studies revealed a left renal mass. He subsequently underwent surgery, and the post-operative pathology confirmed renal cell carcinoma (WHO/ISUP grade 2). Following recovery, the patient began immunotherapy with toripalimab from December 17, 2024, to August 14, 2025. During a routine follow-up appointment while on immunotherapy, the patient's high-sensitivity cardiac troponin I (hs-cTnI) level, measured on a Mindray CL-6000i analyzer, was found to be elevated at 84.4 ng/mL (99th percentile URL = 24.2 ng/mL). This elevation was consistent with previous measurements (Figure 1A). Notably, the patient was asymptomatic, reporting no chest pain, dyspnea, or palpitations. Physical examination was unremarkable. An electrocardiogram and transthoracic echocardiogram performed at the time showed no signs of acute ischemia or structural abnormalities. Given the stark discordance between the significantly elevated hs-cTnI level and the complete lack of clinical evidence for myocardial injury, analytical interference was strongly suspected.

To investigate, a serum sample was subjected to PEG precipitation. Briefly, 100 μ L of the patient's serum was mixed with an equal volume of 25% PEG 6000 solution. The mixture was vortexed, incubated, and then centrifuged at high speed. The hs-cTnI concentration in

the supernatant was measured. The result showed a dramatic decrease to 6.7 ng/mL, corresponding to a recovery rate of 15.9%, which is highly suggestive of macromolecular interference. In contrast, a control sample from a patient with confirmed myocardial infarction showed a recovery of 89.2% (Table 1). Furthermore, serial dilution tests performed on a Mindray CL-8000i analyzer (from 1:10 to 1:50) yielded non-linear results, providing additional support for the presence of an interfering substance (Table 1). Based on these findings, the result was reported with an explanatory note indicating probable macromolecular interference. The patient continued toripalimab treatment without incident, and subsequent hs-cTnI measurements during follow-up remained within the normal range (Figure 1B).

DISCUSSION

This case exemplifies the diagnostic challenge of macromolecular interference causing false-positive hs-cTnI elevation in a patient receiving cancer immunotherapy. The absence of clinical symptoms or corroborating findings on ECG and echocardiogram, in conjunction with a persistently and incongruently high hs-cTnI level, appropriately prompted a laboratory-based investigation for analytical interference. The significant reduction in hs-cTnI after PEG precipitation and the non-linear dilution pattern robustly confirmed the presence of a high-molecular-weight interferent, most likely a macrotroponin complex or heterophilic antibodies [2,5,9].

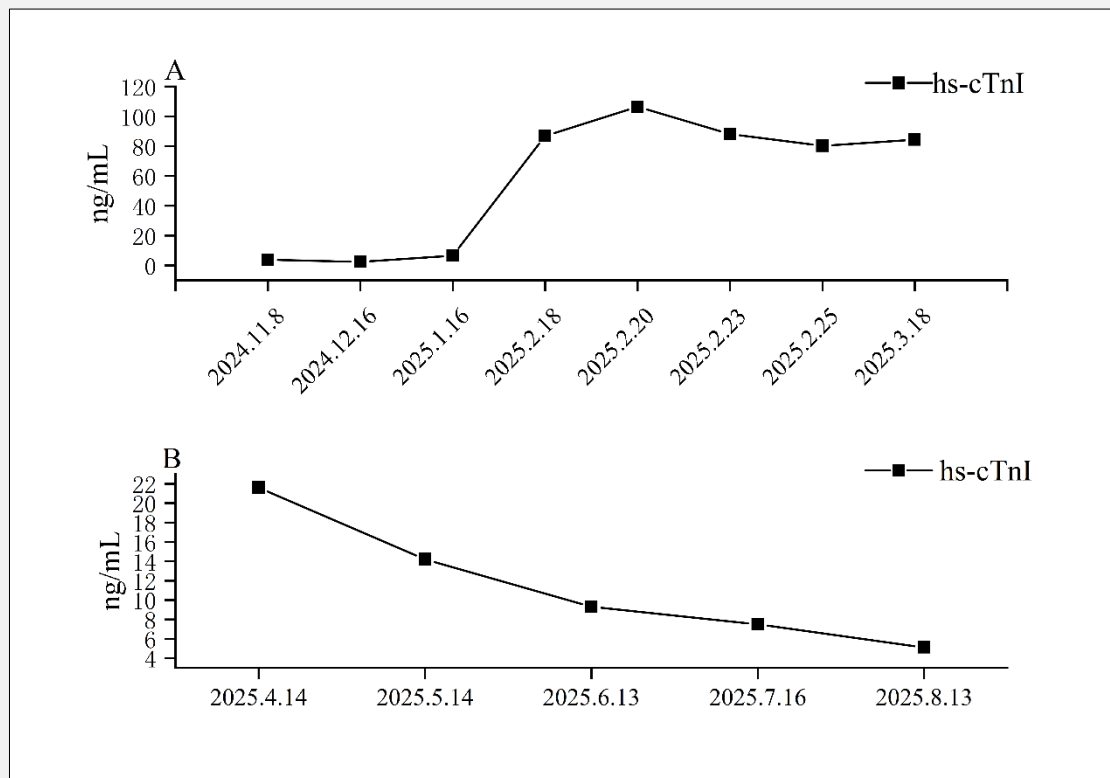
Macromolecular interference in troponin assays is an increasingly recognized clinical dilemma. Macro-troponin, a complex formed between troponin and immunoglobulins, has a prolonged half-life due to reduced renal clearance, leading to persistent false elevations that can last for months or even years [2,6]. This phenomenon can cause considerable diagnostic confusion. Similar cases have been reported in various settings, such as in a young triathlete misdiagnosed with myopericarditis due to macrotroponin-induced cTnI elevation [6], and a middle-aged male with stable hs-cTnI elevations confirmed to be caused by macrotroponin [5]. The persistence of these complexes underscores the importance of considering interference in cases with chronically elevated troponin without a clinical correlate.

In patients undergoing immunotherapy, the situation is particularly complex. While ICI-related myocarditis is a well-documented and life-threatening complication [1, 8], the drugs themselves may also alter the immune landscape in a way that promotes the formation of interfering antibodies or complexes [7]. Toripalimab, an IgG4 monoclonal antibody, could theoretically act as a heterophilic antibody. Alternatively, it may stimulate the production of endogenous anti-drug antibodies or autoantibodies that subsequently interfere with the assay [7]. Therefore, in this patient population, an elevated troponin level, particularly in the absence of typical symptoms, should trigger a dual-approach strategy: a

Table 1. Laboratory investigations for suspected macromolecular interference.

Hs-cTnI PEG 6000 Precipitation Test			
Sample	Original Serum (ng/mL)	Post-PEG (ng/mL)	Recovery (%)
Patient	84.4	6.7	15.9
Control	46.2	20.6	89.2
Serial Dilution Test			
Dilution Factor		Result (ng/mL)	
1 (Neat)		84.0	
10		10.0	
20		6.0	
30		4.0	
40		3.0	
50		2.0	

A PEG recovery rate < 20% is highly suggestive of macromolecular interference. Non-linear reduction upon serial dilution further supports the presence of an interferent.

**Figure 1. A: Displays the initial discordant hs-cTnI elevation, B: Reveals normalized levels post-interference verification.**

thorough clinical assessment for true myocarditis and a parallel laboratory investigation for potential analytical interference [1,7].

Several practical methods are available to laboratories for investigating suspected FPE [9]. Among these, PEG precipitation is a simple, cost-effective, and highly useful screening tool for macrotroponin. A recovery rate of less than 40% is generally suggestive of interference, while a recovery below 20% is considered highly indicative [2,5,9]. The recovery rate of 15.9% in our case is consistent with these findings. Furthermore, non-linearity upon serial dilution and discordant results from an alternative immunoassay platform targeting different epitopes provide additional, corroborating evidence of interference [2,6].

This case underscores the imperative for heightened clinical awareness and robust collaboration between clinicians and laboratory professionals. When troponin elevations are discordant with the clinical picture, clinicians should suspect potential interference and consult with the laboratory [6,9]. Conversely, laboratory professionals must be vigilant when reviewing abnormal results and have established protocols, such as PEG precipitation, for interference testing [9]. The implementation of such standardized procedures is crucial to prevent unnecessary and potentially invasive diagnostic procedures, reduce healthcare costs, and alleviate patient anxiety. As high-sensitivity assays become more pervasive in clinical practice, understanding their limitations, including susceptibility to interference, is paramount for accurate result interpretation and optimal patient management [4,9].

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Declaration of Interest:

The authors declare no conflicts of interest.

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