Clin. Lab. 2026;72:XXX-XXX

CASE REPORT

Rheumatoid Factor Interference in Dual Immunoassays: False-Positive Procalcitonin and CK-MB Results

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SUMMARY

Background: **Immunoassays for procalcitonin (PCT) and CK-MB are critical in diagnosing sepsis and myocardial injury. However, rheumatoid factor (RF), an autoantibody against IgG-Fc, may cause false-positive results by bridging capture and detection antibodies in sandwich immunoassays.**
Methods: **An 81-year-old female with rheumatoid arthritis (RF: 652.0 IU/mL) presented discordant PCT and CK-MB mass results. Interference was investigated via serial dilution, platform comparison (StarPilot immunofluorescence vs. Roche electrochemiluminescence), and RF blocking using IgM-specific inhibitors.**
Results: **StarPilot assays showed elevated PCT (12.38 ng/mL) and CK-MB mass (96.29 ng/mL), contradicting clinical findings (no infection/cardiac symptoms) and CK-MB activity (12 U/L). Ten-fold dilution reduced PCT/CK-MB by 94% and 93%, respectively. Roche platforms returned normal results (PCT: 0.038 ng/mL; CK-MB: 0.203 ng/mL). Adding IgM-blocking reagent (800 µg/mL) normalized PCT (0.023 ng/mL) and CK-MB (0.323 ng/mL).**
Conclusions: **High-titer RF simultaneously interferes with PCT and CK-MB immunoassays. Dilution tests, alternative platforms, and RF blocking are essential to mitigate misdiagnosis. Laboratories should implement RF interference protocols for discordant results.**
(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.250666)

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KEYWORDS

rheumatoid factor, immunoassay interference, false-positive, procalcitonin, CK-MB

INTRODUCTION

Procalcitonin (PCT) and CK-MB mass are vital biomarkers for bacterial sepsis and acute myocardial infarction, respectively. PCT, a glycoprotein precursor of calcitonin, rises significantly during systemic bacterial infections but remains low (< 0.06 ng/mL) in healthy individuals [1]. CK-MB mass, a cardiac-specific isoform of creatine kinase, is released after myocardial injury [2].

Immunoassays using sandwich antibody designs (e.g., enzyme-linked immunosorbent assay, chemiluminescence) are susceptible to interference from heterophilic antibodies, particularly rheumatoid factor (RF) [3,4]. RF binds nonspecifically to the Fc region of animal-derived antibodies used in assays, forming false bridges

Case Report accepted June 26, 2025

Table 1. Initial and serial laboratory findings.

Feature	Value	Reference range
Initial admission test results		
PCT	**8.8 ng/mL**	**< 0.06 ng/mL**
CK-MB mass	**80.89 ng/mL**	**0 - 5.86 ng/mL**
Serial monitoring results		
PCT - day 2	**10.47 ng/mL**	**< 0.06 ng/mL**
PCT - day 3	**12.38 ng/mL**	**< 0.06 ng/mL**
CK-MB mass - day 3	**96.29 ng/mL**	**0 - 5.86 ng/mL**
Supporting biochemical findings		
CK-MB activity (admission)	**12 U/L**	**0 - 24 U/L**
Troponin I (admission)	**0.03 µg/L**	**0.00 - 0.10 µg/L**
Myoglobin (admission)	**72.3 µg/L**	**< 100 µg/L**
Creatine Kinase (admission)	**25 U/L**	**40 - 200 U/L**

Table 2. Summary of interference testing for PCT and CK-MB in an RF-positive patient.

Test type	Sample/condition	CK-MB (ng/mL)	PCT (ng/mL)
Re-tested results (day 3)	**original sample (StarPilot platform)**	**61.00**	**7.79**
Dilution test	**10-fold dilution (StarPilot platform)**	**4.22**	**0.49**
Platform comparison	**Roche cobas e601 platform**	**0.203**	**0.038**
Interference screening	**+ 1 mg/mL polymeric antibody**	**–**	**5.47**
	+ 1 µg/mL fluorescein antibody	**–**	**5.23**
	+ 100 µg/mL sheep IgG	**–**	**5.10**
	+ 400 µg/mL IgM-blocking reagent	**–**	**2.13**
RF blocking optimization	**+ 400 µg/mL IgM-blocking reagent**	**–**	**2.28**
	+ 600 µg/mL IgM-blocking reagent	**–**	**2.191**
	+ 800 µg/mL IgM-blocking reagent	**0.323**	**0.023**

that generate spurious signals [5]. While manufacturers incorporate blocking agents to neutralize RF, high-titer or atypical RF subtypes may evade inhibition [6,7]. We report a case of dual PCT and CK-MB false positivity due to RF interference, highlighting diagnostic challenges and resolution strategies.

CASE PRESENTATION

An 81-year-old woman with rheumatoid arthritis, hypertension, and diabetes mellitus was admitted to the endocrinology-rheumatology unit for management of her autoimmune condition. During routine admission testing, markedly elevated levels of procalcitonin (PCT: 8.8 ng/mL; reference range < 0.06 ng/mL) and CK-MB mass (80.89 ng/mL; reference range 0 - 5.86 ng/mL) were detected using the StarPilot immunofluorescence platform (PII0056, Shenzhen, China). These results were discordant with the clinical presentation, as the patient exhibited no signs of infection (e.g., fever, leukocytosis) or cardiac injury. Further biochemical testing revealed normal CK-MB activity (12 U/L; reference range 0 - 24 U/L), troponin I (0.03 µg/L), myoglobin (72.3 µg/L), and creatine kinase (25 U/L) (Table 1).

Serial monitoring demonstrated persistently elevated PCT (Day 2: 10.47 ng/mL; Day 3: 12.38 ng/mL) and CK-MB mass (Day 3: 96.29 ng/mL) on the StarPilot platform, while other cardiac markers remained within normal limits (Table 1). The contradiction between elevated CK-MB mass and normal CK-MB activity, coupled with the absence of clinical correlation, prompted an interference investigation.

Due to limited sample volume, initial interference

screening was performed only for PCT. The day 3 sample (re-tested PCT: 7.79 ng/mL; CK-MB mass: 61.00 ng/mL) was used for interference studies (Table 2). Addition of polymeric antibodies (1 mg/mL), fluorescein antibodies (1 µg/mL), or sheep IgG (100 µg/mL) did not significantly reduce PCT levels (5.47, 5.23, and 5.10 ng/mL, respectively), excluding common heterophilic antibodies as the primary interferent.

Ten-fold dilution reduced PCT from 7.79 ng/mL to 0.49 ng/mL (94% decrease) and CK-MB mass from 61.00 ng/mL to 4.22 ng/mL (93% decrease), indicating analytical interference. Retesting on a Roche cobas e601 electrochemiluminescence platform returned normal results (PCT: 0.038 ng/mL; CK-MB mass: 0.203 ng/mL). Subsequent studies identified RF as the interferent: Adding IgM-specific blocking reagent (800 µg/mL) normalized PCT (0.023 ng/mL) and CK-MB mass (0.323 ng/mL) (Table 2). The patient's RF level was 652.0 IU/mL (ref. 0 - 30 IU/mL) via immunonephelometry (Beckman IMMAGE 800). After excluding true pathology, the patient was discharged without therapy.

DISCUSSION

Rheumatoid factor (RF) represents a group of autoantibodies reactive to the Fc portion of human IgG. It encompasses five immunoglobulin subclasses: IgA, IgD, IgE, IgG, and IgM, with IgM being the predominant isotype. RF has been reported to cause erroneous results in numerous types of immunoassays, including enzyme-linked immunosorbent assays (ELISA), chemiluminescent microparticle immunoassays (CMIA), particle-enhanced immunonephelometric assays, antibody-conjugated magnetic immunoassays (ACMIA), and, as reported by the authors, immunofluorescence assays [8,9]. Furthermore, immunoassays utilizing both polyclonal and monoclonal antibodies have been demonstrated to be susceptible to interference by RF [10]. One reason for RF's broad impact is its ability to cause interference through various mechanisms, including acting as an anti-human antibody, a heterophilic antibody, and an anti-animal antibody [11,5].

In this case, the source of interference, RF, was identified through interference screening tests. To mitigate interference from RF and other heterophilic antibodies, most modern immunoassays utilize non-specific immunoglobulins, similar to the IgM-specific blocking reagent we employed to neutralize the interfering antibodies. Manufacturers continuously strive to optimize their assays by incorporating blockers into the reagents; for instance, StarPilot reagents contain heterophilic antibody blockers. However, interference can still occur occasionally, even in assays employing sufficient blockers, because RF and other heterophilic antibodies are inherently heterogeneous. Certain patients may harbor unique antibody subclasses that might not be neutralized by non-specific immunoglobulins [4,7]. In this case, adding an IgM-specific blocking reagent at 800 µg/mL to either the sample or the reagent successfully blocked the RF interference.

In immunological testing, encountering abnormally elevated results for biomarkers like PCT or CK-MB that contradict the clinical presentation (e.g., absence of infection or myocardial injury symptoms) should raise suspicion of interference from factors such as rheumatoid factor (RF). Key indicators for recognition include a significant decrease upon retesting diluted samples (e.g., PCT dropping from 7.79 ng/mL to 0.49 ng/mL) and substantial discrepancies when using alternative testing platforms (e.g., false positives on StarPilot versus negative results on Roche). Verification methods involve adding IgM-specific blocking reagents (e.g., PCT decreasing to 0.023 ng/mL with 800 µg/mL) and measuring the patient's RF level (which was 652.0 IU/mL in this case).

Preventive measures are recommended: selecting chemiluminescence platforms known for strong interference resistance, establishing retesting protocols involving dilution or blocking reagents, enhancing clinical communication (e.g., flagging results for patients with high RF levels), and providing regular training for laboratory personnel to recognize interference patterns. Through multidisciplinary collaboration and standardized operating procedures, the risk of false positives can be minimized, ensuring the delivery of accurate and reliable test results.

Sources of Support:
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for profit sectors.

Declaration of Interest:
All authors declare that they have no competing interests.

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