False-positive semiquantitative immunochromatography assays for procalcitonin in three patients with rheumatoid arthritis—A case series

Hiroe Sato¹ | Satoshi Ito² | Kiyoshi Nakazono² | Yoichi Kurosawa³ | Yukiko Nozawa³ | Takeshi Nakatsue³ | Yoko Wada³ | Takeshi Kuroda¹ | Yoshiki Suzuki¹ | Masaaki Nakano⁴ | Ichiei Narita³

¹Niigata University Health Administration Center, Niigata City, Japan
²Department of Rheumatology, Niigata Rheumatic Center, Shibata City, Japan
³Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata City, Japan
⁴Department of Medical Technology, School of Health Sciences, Faculty of Medicine, Niigata University, Niigata City, Japan

Correspondence
Hiroe Sato, Niigata University Health Administration Center, 2-8050 Ikarashi, Nishi-ku, Niigata City, 950-2181, Japan.
Email: hiroes@med.niigata-u.ac.jp

Abstract
We report three rheumatoid arthritis (RA) patients with false-positive procalcitonin (PCT) based on semiquantitative immunochromatography assays without infection, but who had negative PCT assay results based on quantitative methods. Immunochromatography was useful for screening; however, other heterophilic antibodies rather than rheumatoid factor were possible to affect, especially in RA flare.

Keywords
heterophilic antibody, immunochromatography assay, procalcitonin, rheumatoid arthritis, rheumatoid factor

1 | INTRODUCTION

Serum procalcitonin (PCT) levels are now widely used as a marker of bacterial infection. Serum PCT is elevated by IL-1β and TNF associated with systemic inflammation. We previously reported that the serum PCT level was a specific, but not a sensitive, marker for detecting bacterial infection in patients with rheumatoid arthritis (RA).¹ In that study, we used a quantitative method to measure PCT levels in stored serum. Semiquantitative immunochromatography assays are widely used for detecting various kinds of infection, tumor markers, and hormones because results can be obtained rapidly and easily. An immunochromatography assay for detecting PCT has also been developed. After the measurement of PCT was approved in Japan in 2006, our hospitals adopted an immunochromatography method in daily practice, because it takes only 30 minutes to see results after adding a patient’s serum to the kit. However, a dissociation between the PCT results obtained using the semiquantitative immunochromatography assay and patient clinical features was seen in three patients with RA, in whom the PCT levels were ultimately determined to be normal according to a quantitative method.

Here, we report three patients with RA who had false-positive PCT results using a semiquantitative immunochromatography method but negative using a quantitative method, who did not associate with any infectious diseases.
2 | CASE PRESENTATION

2.1 | Case 1

A 48-year-old man, who had been diagnosed with RA 9 months earlier and was treated with methotrexate (MTX) 10 mg per week, bucillamine 200 mg per day, and prednisolone (PSL) 5 mg per day, was started on infliximab (IFX) because of high disease activity. However, the IFX therapy was not effective and thus was changed to etanercept (ETN) after two doses of IFX. After two ETN injections, he developed a high fever. His C-reactive protein (CRP) level was 5.3 mg/dL; the rheumatoid factor (RF) level was 758 IU/mL, and the immunoglobulin G (IgG) level was 1090 mg/dL. Firstly, the infectious disease was suspected and laboratory tests were performed. The PCT level measured using an immunochromatography assay was 0.5-2 ng/mL (PCT-Q®; BRAHMS Aktiengesellschaft) (normal range: <0.5 ng/mL). Finally, no infection was apparent and a flare-up of RA was suspected. Tocilizumab was started, and the fever and CRP level improved. Later, a quantitative examination (chemiluminescent enzyme immunoassay; CLEIA) was performed using stored sample and was negative for PCT (≤0.1 ng/mL) (SphereLight PCT®; Wako Pure Chemical Industries).

2.2 | Case 2

In a 62-year-old woman had been diagnosed with RA 10 years earlier and treated with MTX 6 mg per week, RA disease activity was high and we started IFX. This proved temporarily effective but her disease activity flared. The CRP, RF, and IgG levels were 2.0 mg/dL, 34 IU/mL, and 1176 mg/dL, respectively. The PCT level measured with an immunochromatography assay was 0.5-2 ng/mL. No infection was apparent, and a flare-up of RA was suspected. She was started on ETN, and the CRP level improved. Later, PCT was measured using stored sample and was negative on a quantitative electrochemiluminescence immunoassay (ECLIA) (ELECSYS BRAHMS PCT assay, Cobas6000; Roche) (0.04 ng/mL). One year later, the PCT level was negative (≤0.5 ng/mL) according to the immunochromatography assay. The RF and IgG levels had not changed and were 16 IU/mL and 1220 mg/dL, respectively.

2.3 | Case 3

An 80-year-old man, who had been diagnosed with RA 10 years earlier and treated with MTX 8 mg per week and PSL 10 mg per day, was admitted to our hospital with septic shock and treated successfully. His PCT level at admission was 10 ≤ ng/mL. However, the PCT level determined by immunochromatography was still 10 ≤ ng/mL after the infection had been improved. We examined PCT by quantitative examination using ECLIA (ELECSYS BRAHMS PCT assay, Cobas6000; Roche), and the result was negative for PCT (0.02 ng/mL). Laboratory tests showed CRP 1.4 mg/dL, RF 511 IU/mL, and IgG 885 mg/dL.

2.3.1 | PCT levels measured by immunochromatography assay and RF levels

Niigata Rheumatic Center used an immunochromatography assay to measure PCT from January 2009 to January 2013. The PCT level was measured in total 436 patients, of whom 332 also had their RF level measured within 1 month before or after the PCT measurement. Total 31 patients (9.3%), including Case 1, had an RF level ≥500 IU/mL, of whom the PCT level was <0.5 ng/mL in 11 (35.5%), 0.5-2 ng/mL in 10 (32.3%), 2-10 ng/mL in 6 (19.4%), and > 10 ng/mL in 4 (12.9%) (Figure 1-A). Of the patients with a PCT level
<0.5 ng/mL (n = 232), only 5% had an RF level ≥500 IU/mL, with most having an RF level <500 IU/mL (Figure 1B). Four of patients with negative PCT had an RF level >2000 IU/mL, and the maximum RF level was >4000 IU/mL.

3 | DISCUSSION

The three RA patients described herein were positive for PCT on a semiquantitative immunochromatography assay, without any infectious diseases or even after sepsis had been improved: the results with quantitative methods were negative. In the manufacturer’s documentation (supplied with the PCT-Q® semiquantitative immunochromatography kit), it is stated that high RF levels and heterophilic antibody may cause false-positive results. Our data suggest that a high RF level does not usually influence the measurement of PCT by immunochromatography assay, because a higher RF level (500 ≤ IU/mL) was more frequently observed in association with a lower PCT level measured by immunochromatography assay. At present, our hospitals measure PCT using a quantitative method, because the lower value below 0.5 ng/mL was not determined using a semiquantitative method and a visual check of the test line was sometimes difficult and errors could be concerned.

Rheumatoid factor is a heterophilic antibody, and high RF titers are reported to interfere with immunometric assays. RF reacts with the Fc portion of rabbit IgG and human IgG. Two of our patients, one and three, had RF levels greater than 500 IU/mL, which might have interfered with the immunochromatography assay. However, a high RF titer does not always cause a false-positive PCT-Q® result. The immunochromatography assay was considered useful for screening, even in patients with RA.

There have been several reports of analytical interference from human anti-mouse antibodies (HAMA) with PCT-Q® results; HAMA are major heterophilic antibodies that react with animal immunoglobulin. A 55-year-old woman had a positive result for HAMA on PCT-Q®, but was negative according to a quantitative method (KRYPTOR® BRAHMS®). After adding mouse IgG to the patient’s serum, the PCT-Q® result for PCT also became negative. In another reported case, analytical interference decreased after adding goat serum, mouse serum, or goat IgG. Immunochromatography assays like PCT-Q® are based on a two-site immunoassay done using animal antibodies. According to PCT-Q®, antikatacalcin antibody labeled with gold colloid (mouse monoclonal antibody) reacts with serum PCT. The bound substance then moves to the test line via the capillary effect. On the test line, antihuman calcitonin antibody (sheep polyclonal antibody) reacts with the serum PCT and produces a color, while on the control line, anti-mouse IgG antibody (goat) reacts with the gold colloid-labeled antibody and produces a color. The mechanism underlying the false-positive caused by HAMA and other heterophilic antibodies is thought to be their binding to, or cross-reaction with, the antikatacalcin antibody labeled with gold colloid (mouse monoclonal antibody) and antihuman calcitonin antibody (sheep polyclonal antibody) on the test line. When two monoclonal mouse antibodies are used in a single assay, the frequency of false-positive results increases substantially vs use of a mouse monoclonal antibody and a polyclonal antibody from another species.

Heterophilic antibody interference has been reported for many tumor markers (alpha-fetoprotein, CA125, beta-human chorionic gonadotropin, squamous cell carcinoma antigen, and prostate-specific antigen), hormones (thyrotrophic hormone and estradiol), and infection markers (influenza and human hepatitis B antigen) using immunochromatography assays. Commercial quantitative methods have been improved to reduce heterophilic interference by removing the Fc portion and avoid the binding of heterophilic antibodies; this can also be achieved by adding a HAMA blocker or adsorbing the heterophilic antibodies using IgG from the same animal or serum, as a secondary antibody. In comparison, most immunochromatography assays, including PCT-Q®, use whole immunoglobulins, which result in more frequent heterophilic interference. Immunochromatography assays are easy, rapid, and widely used. However, heterophilic antibody interference is a concern.

The reported prevalence of HAMA was 5.0 ~ 8.3% for both healthy subjects and patients with autoimmune thyroid disease. Interfering antibodies were detected in 40% of the healthy subjects, while the incidence of immunoassay interference was lower in that population. Heterophilic antibody production is thought to result from contact with animals, the administration of animal-derived biological agents, vaccines, infection, or blood transfusions. HAMA was reported to cause false-positive CA125 in patients with ovarian cancer treated with mouse monoclonal antibody. Chimeric or humanized biologics are thought to confer a lower risk of HAMA development than mouse monoclonal antibodies. However, antidrug antibodies, including human antichimeric antibody (HACA), are often produced and cause secondary drug failure in patients with RA and inflammatory bowel disease. Such antidrug antibodies are believed to interfere with immunoassay results in some patients. Two of our patients, one and two, failed to respond to IFX therapy, and interference caused by HACA was suspected. In patient 2, the false-positive reaction was eliminated after successful ETN treatment following the secondary failure of IFX. Generally, antidrug antibodies are produced more frequently during RA flares, and controlling RA activity with ETN might reduce the production of heterophilic antibodies; further study of this is needed. Methods to reduce interference in chromatography assays of patients with RA are needed, such as using commercial HAMA blockers.
One major limitation of this study is that we did not examine whether adding IFX or mouse serum to patients' serum improves the false-positive result. We could not conduct further studies because the serum samples were inadequate.

In conclusion, we herein reported three patients with RA who had false-positive results for PCT measured using a semiquantitative immunochromatography assay without infection. Heterophilic antibodies (ie, higher RF levels and HAMA) were believed to have interfered with the two-site immunochromatographic method done using animal immunoglobulin, including the Fc portion. However, a higher RF level usually did not affect the PCT level determined by immunochromatography assay. Patients with RA sometimes have antidrug antibodies, as induced by biological agents, and these could be heterophilic antibodies. Quantitative methods other than semiquantitative immunochromatography assays should be used in patients with RA.

ACKNOWLEDGMENT
None.

CONFLICT OF INTEREST
None.

AUTHORS' CONTRIBUTION
HS, SI, KN, YK,YN, TN, and YW helped to collect, analyze, and interpret the data. HS wrote the initial draft of the manuscript. TK, YS, MN, and IN assisted in the preparation of the manuscript. All of the authors have critically reviewed the manuscript. All authors approved the final version of the manuscript.

PATIENT CONSENT
Written informed consents for publication were obtained from the patients.

ORCID
Hiroe Sato https://orcid.org/0000-0002-3952-7163
Takeshi Kuroda https://orcid.org/0000-0001-8246-0171

REFERENCES
1. Sato H, Tanabe N, Murasawa A, et al. Procalcitonin is a specific marker for detecting bacterial infection in patients with rheumatoid arthritis. J Rheumatol. 2012;39:1517-1523.
2. Bolstad N, Warren DJ, Nustad K. Heterophilic antibody interference in immunometric assays. Best Pract Res Clin Endocrinol Metab. 2013;27:647-661.
3. Olsson P, Theander E, Bergstrom U, et al. Multiplex cytokine analyses in patients with rheumatoid arthritis require use of agents blocking heterophilic antibody activity. Scand J Rheumatol. 2017;46:1-10.
4. Desideri-Vaillant C, Rouby Y, Cardon N, et al. Analytical interference in determination of procalcitonin by PCT-Q (Brahms). Pathol Biol (Paris). 2006;54:293-295.
5. Desvignes P, Boutib A. Semi quantitative determination of blood procalcitonin by the immunochromatographic BRAHMS PCT-Q test: false positive result. Ann Biol Clin (Paris). 2005;63:358-359.
6. Warade J. Retrospective approach to evaluate interferences in immunoassay. EJIFCC. 2017;28:224-232.
7. Yang X, Ji H, Lin X, et al. Detection and elimination of interference by the heterophilic antibody in antibody microarray-based immunoassay. Clin Chem. 2011;57:527-529.
8. Oei AL, Sweep FC, Massuger LF, et al. Transient human anti-mouse antibodies (HAMA) interference in CA 125 measurements during monitoring of ovarian cancer patients treated with murine monoclonal antibody. Gynecol Oncol. 2008;109:199-202.
9. Soleimanpour SA. Fulminant liver failure associated with delayed identification of thyroid storm due to heterophile antibodies. Clin Diabetes Endocrinol. 2015;1:12.
10. Guo CY, Tang YG, Qi ZL, et al. Development and characterization of a panel of cross-reactive monoclonal antibodies generated using H1N1 influenza virus. Immunobiology. 2015;220:941-946.
11. Itakna M, Kitahama S, Fukasawa N, et al. Incidence of anti-mouse IgG in normal subjects and patients with autoimmune thyroid disease. Intern Med. 1992;31:984-988.
12. Kricka LJ. Human anti-animal antibody interferences in immunochemical assays. Clin Chem. 1999;45:942-956.
13. Maloney DG, GrilloLopez AJ, White CA, et al. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin’s lymphoma. Blood. 1997;90:2188-2195.
14. van der Bijl AE, Breedveld FC, Antoni CE, et al. An open-label pilot study of the effectiveness of adalimumab in patients with rheumatoid arthritis and previous infliximab treatment: relationship to reasons for failure and anti-infliximab antibody status. Clin Rheumatol. 2008;27:1021-1028.
15. Afif W, Loftus EV Jr, Faubion WA, et al. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. Am J Gastroenterol. 2010;105:1133-1139.

How to cite this article: Sato H, Ito S, Nakazono K, et al. False-positive semiquantitative immunochromatography assays for procalcitonin in three patients with rheumatoid arthritis—A case series. Clin Case Rep. 2020;8:1704–1707. https://doi.org/10.1002/ccr3.2968