博士学位論文

免疫比濁法（Turbidimetric Immunoassay：TIA）で測定されたIgG結合（親和性）IgM型M蛋白の偽低値発生機序に関する研究

井本　真由美

Doctoral Dissertation

Study on the Mechanism of False Low Measurement of IgG-Binding (Affinity) IgM Type M Protein by Turbidimetric Immunoassay

February 2023

Mayumi Imoto

課博 ・ (論博)

同　意　書

令和5年 2月 10日

近畿大学大学院
医学研究科長　殿

共著者 上砥 修法 (印)　　共著者 渡辺 勝紀 (印)

共著者 山田 俊幸 (印)　　共著者 _____ (印)

共著者 _____ (印)　　共著者 _____ (印)

共著者 _____ (印)　　共著者 _____ (印)

共著者 _____ (印)　　共著者 _____ (印)

論文題目

Study on the Mechanism of False Low Measurement of IgG-Binding (Affinity) IgM Type M Protein by Turbidimetric Immunoassay

下記の博士論文提出者が、標記論文を貴学医学博士の学位論文（主論文）として使用することに同意いたします。

また、標記論文を再び学位論文として使用しないことを誓約いたします。

記

1．博士論文提出者氏名　　井本　真由美

2．専攻分野　　医学系　　臨床検査医学

Clin. Lab. 2022;68:2517-2525

ORIGINAL ARTICLE

Study on the Mechanism of False Low Measurement of IgG-Binding (Affinity) IgM Type M Protein by Turbidimetric Immunoassay

Mayumi Imoto [1], Toshinori Kamisako [2], Katsunori Watanabe [3], Toshiyuki Yamada [4]

[1] *Department of Clinical Laboratory, Kindai University Hospital, Osakasayama, Osaka, Japan*
[2] *Department of Clinical Laboratory Medicine, Kindai University Faculty of Medicine, Osakasayama, Osaka, Japan*
[3] *Eiken Chemical, Biochemical Research Laboratory-I, Nogi, Tochigi, Japan*
[4] *Department of Clinical Laboratory Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan*

SUMMARY

Background: **The purpose of this study was to investigate the immunological and physical characteristics of IgM-λ type M-protein from patients who were measured low in the turbidimetric immunoassay (TIA) IgM assay without error codes for high concentration to determine the cause of the false low levels and to clarify the mechanism of their occurrence.**

Methods: **Materials were IgM patient samples and 8 serum samples from other IgM M-protein patients as controls. Patient samples were assayed by the TIA method, in which five manufacturers and six models (two reagent manufacturers) share the principle, and the BN ProSpec method (nephelometric method), which has a different principle. Dilution linearity tests, IgG addition experiments, isoelectric point electrophoresis, and hydrophobic chromatography were performed on patients and subjects. In addition, the binding capacity of γ-globulin by BIACORE was also examined.**

Results: **The reaction curve of the patient IgM curved downward when the concentration of IgM exceeded 20 g/L, and no error code was obtained. In the measurement by the TIA method of five manufacturers and six models, patient IgM was measured at a false low level with no error code obtained in undiluted dilution by any of the instruments and reagents, but could be measured without any problem by the nephelometric method. In addition, in the patient IgG addition experiment, only patient IgM showed a false low level under high IgG concentration. Furthermore, the binding capacity of patient IgM to γ-globulin (IgG) by BIACORE was significantly higher than that of the control IgM-type M protein.**

Conclusions: **Patient IgM has an affinity (binding capacity) for IgG and forms an IgM-IgG complex under conditions of high IgG concentration. It was speculated that this complex inhibited the reaction with the anti-IgM antibody and the absorbance of the second reaction did not increase, suggesting a false low.**

(Clin. Lab. 2022;68:2517-2525. DOI: 10.7754/Clin.Lab.2022.220203)

Correspondence:
Mayumi Imoto
Department of Clinical Laboratory
Kindai University Hospital
377-2 Osakasayama
Osaka, 589-8511
Japan
Phone: + 81 72-366-0221
Fax: + 81 72-366-0206
Email: maimoto@zmail.plala.or.jp

KEYWORDS

turbidimetric immunoassay (TIA), false low level, error code, monoclonal protein, system of laboratory examination

LIST OF ABBREVIATIONS

TIA - turbidimetric immunoassay
IEP - immunoelectrophoresis

Manuscript accepted April 20, 2022

INTRODUCTION

Immunoassay is a useful method for the detection of a trace substance in clinical samples, but certain substances (proteins in many cases) in blood samples cause nonspecific reactions in the reaction system and influence the measurement result. A falsely high result caused by human anti-mouse antibody (HAMA) was initially reported as a nonspecific reaction in immunoassay in an hCG measurement system in 1985 [1]. After that, false high and low levels caused by substances other than HAMA, such as heterophilic antibody [2], rheumatoid factor [3], and M protein (monoclonal protein) [4,5], have been reported. To improve the measurement system to reduce such nonspecific reactions in immunoassays, several blocking agents and reducing agents have been added as a prevention method [6]. However, measurement devices are capable of only displaying an error code informing of a high concentration, so that laboratory technologists need to discover nonspecific reactions at the time of the test item measurement based on the following items: 1) Abnormally high or low measured value [7], 2) negative value [8], 3) checking by comparison with other items, 4) inconsistency with clinical findings.

Though nephelometry [9], latex agglutination [10], and turbidimetric immunoassay (TIA) are available for immunoglobulin measurement [11], TIA is frequently used because of its low operational cost [12]. Immunoglobulin measuring instruments display an 'error code' in the following conditions: 1) The immunoglobulin level is high, 2) white turbidity occurs in the reaction with polyethylene glycol (PEG) in the first reaction, and the abnormality is discovered by confirming the reaction curve plotting measurement points and changes in the absorbance. Therefore, so far, we have presumed that no erroneous report of immunoglobulin measurement by TIA if we did not miss abnormal values and the error code.

In this study, we discovered IgM-type M protein, which caused a falsely low level of IgM without error notification in IgM measurement by TIA. We reported the case in a Japanese Journal named "Rinsho Byori (in Japanese)" as a preliminary case report in 2018, in which we only investigated and discussed the routine laboratory test results. To investigate the cause of this false low level (developmental mechanism), we analyzed the immunochemical and physicochemical characteristics of IgM-type M protein of the patient using samples of other patients with IgM-type M protein as a control.

Case

This patient was a female in her 60s with Waldenstrome's Macroglobulinemia. The main test findings were: total protein (TP),10.0 g/dL; ALB, 2.6 g/dL; CRP, 1.4 mg/dL; Hb, 9.8 g/dL. Polyclonal hypergammaglobulinemia and the presence of 32.7% (32.7 g/L) M protein at β position were detected in serum protein fraction (Figure 1A), and IgM λ-type M protein was detected at β position by immunoelectrophoresis (IEP) (Figure 1B). The immunoglobulin levels measured by TIA (Cobas-8000, c702, Roche) were: IgG, 32.57 g/L; IgA, 2.58 g/L; IgM, 2.28 g/L. Cryoglobulin, Sia test (spot immunoprecipitate assay), and qualitative urinary Bence Jones Protein (BJP) test were negative. Since there were deviations among the IEP findings, M protein level in the serum M protein fraction, and IgM level were measured by TIA, the serum was 10-fold diluted and measured by TIA and the IgM level was 38.38 g/L. When it was measured without dilution, a 'high concentration error code' was not displayed and no influence of turbidity was observed in the reaction curve.

MATERIALS AND METHODS

Materials and immunoglobulin measurement devices

Eight patient serum samples with IgM-type M protein for which consent to the use of the residual samples after completion of the tests was obtained were used as a control. The immunoglobulin measurement device used by our laboratory is the Cobas8000 c702 module of Roche Diagnostics K.K. (Roche) and the measurement reagent is 'Auto Wako IgM • N' (Fuji Film Wako Pure Chemical Corporation). In this study, immunoglobulin was measured using Cobas8000 (Roche), H-7180 (Hitachi High-Tech Corporation), BM 6050 (JEOL Ltd.), c16000 (Canon Medical Systems), AU640 (Beckman Coulter Inc.) for the measurement device and 'Autowako IgM • N'. In addition, immunoglobulin was measured using BM8030 (JEOL Ltd.) for the measurement device and 'N-Assay TIA IgM-SH Nittobo' (Nittobo Medical Co., Ltd.) for the reagent. Furthermore, immunoglobulin was measured using a measurement device of nephelometry, BN ProSpec (Siemens Healthcare Diagnostics, K.K. (Siemens)) and 'N-anti- serum IgM' (Siemens). The serum protein fraction was measured using Epalyzer 2 Junior (Helena Laboratories). IEP was performed employing the conventional method using an agarose plate for IEP (Helena Laboratories). The antibodies and γ-globulin used were products of Helena Laboratories and Sigma-Aldrich Co. LLC, respectively. It has been confirmed that the concentrations of IgG, IgA, and IgM in 10 g/L human γ-globulin solution are 6.91, 0.22, and 0 g/L, respectively.

Methods

Confirmation of dilution linearity and display of error code: Ten-fold serial dilution systems of the patient and control samples (sample 7: IgG, 11.49 g/L; IgA, 0.46 g/L; IgM, 41.50 g/L) were prepared with saline and the measured concentrations and the presence or absence of an error code display were observed.

Measurement using other measurement devices: The patient serum was subjected to measurement using 6 TIA-based models of 5 manufacturers and nephelometry-based BN ProSpec.

Gamma globulin addition experiment: Gamma globulin was added to the patient serum in which IgM was decreased by treatment and the undiluted sample got an error message and 7 samples of IgM-type M protein in which the undiluted sample got an error message (final IgG concentration: 29.78 - 42.7 g/L), and whether the addition influences the measured value of IgM was observed.

Comparison of ammonium sulfate recovery rate: Since a difference in the ammonium sulfate fraction recovery rate was noted between the patient and control samples in the patient IgM purification process, the ammonium sulfate recovery rate was compared between the patient sample and 7 IgM-type M protein samples. As shown in Figure 2 left flowchart, ammonium sulfate treatment was performed as follows: The patient and control sera were diluted 2-fold with PBS, mixed with 500 µL of saturated ammonium sulfate at 1:1, kept standing for 24 hours at 4°C, and centrifuged at 10,000 rpm for 15 minutes. The supernatant was collected, washed with 500 µL of 50% ammonium sulfate, and centrifuged again at 10,000 rpm for 15 minutes, and the γ globulin fraction was acquired.

Findings of isoelectric focusing: After ammonium sulfate treatment, the patient and control sera were applied to gel filtration chromatography following Figure 2 right flowchart and the IgM fraction was collected. The IgM fraction was concentrated using Amicon 100K, and the IgM concentration was measured by TIA, adjusted to 0.5 mg/mL, and used as a sample. The isoelectric point was compared using Phast System (GE).

Hydrophobic interaction chromatography findings: HPLC Prominence (SHIMADZU) was used. The patient and 6 control samples purified by ammonium sulfate fractionation and gel filtration chromatography were applied to hydrophobic interaction chromatography. In addition, albumin-removed samples of the patient and control sample ⑦ were prepared using Econo-Pac Serum IgG Purification Column (DEAE Affi-Gel blue gel, BIO-RAD) and analyzed following the package insert.

Analysis of binding to γ-globulin using BIACORE: Using a device analyzing the intermolecular interaction, BIACORE (GE), the patient serum and partially purified sample after ammonium sulfate fractionation were added to the sensor chip to which γ-globulin collected from healthy subjects was immobilized, and binding to γ-globulin was measured. Six control samples were similarly measured.

RESULTS

Confirmation of dilution linearity and error code display

In the control samples, an error code notifying of a high level was displayed for the original sample and a high concentration range at a low dilution rate in all dilutions excluding 1/10. However, in the patient sample, the value was 40.5 g/L at a dilution rate of 10-fold (1/10), but the error message notifying of a high level was displayed only up to 2-fold, and no error code was displayed in the higher concentration range which was measured as 2.0 - 4.0 g/L false low level (Figure 3A). The reaction curve of the patient serum measured using cobas is shown in Figure 3B.

The absorbance did not increase in the original sample and was measured as a low level, but the absorbance increased when the serum was diluted 2, 5, 10, 20, and 40-fold and showed an error code (Figure 3B).

Measurement using other measurement devices

None of the other TIA-based measurement devices (6 models of 5 manufacturers) displayed an error code for the undiluted sample. Regarding the measurement reagent, the results were the same when a reagent of N Company was used. However, using nephelometry-based BN ProSpec, IgM was measured as 35.7 g/L (Table 1).

Gamma globulin addition experiment

Gamma globulin was added to the 7 samples of IgM-type M protein getting an error code in the measurement of undiluted samples, the final IgG concentration was adjusted to 30.0 - 40.0 g/L, and IgM was measured. All samples showed an error code. On the other hand, when γ-globulin was added to the sample which resulted in getting an error code which may have been due to decreased in IgG and IgM by treatment, the error code was again not displayed (not shown in Table).

Comparison of the ammonium sulfate fraction recovery rate

The IgM recovery rate was 70% or higher in all control samples excluding the sample of the Sia test-positive patient. In the patient sample, the recovery rates of IgG and IgA were 75.4 and 72.8%, respectively, showing as high a recovery rate as those in the control samples, but the IgM recovery rate was 40.0 - 47.8%, being low, and only the IgM recovery rate was decreased. In analysis of the ammonium sulfate fraction of the patient sample shown in Figure 4, the measured values of IgG, IgA, and IgM after treatment with ammonium sulfate showed dilution linearity. Almost no globulin component was detected in the supernatant after treatment with ammonium sulfate in analysis using protein fraction electrophoresis. Large amounts of IgG and IgM were detected in the ammonium sulfate-treated sample. Regarding the recovery rate, the experiment was repeated several times, but the IgM recovery rate was low (Figure 4).

Findings of isoelectric focusing

In control samples ① - ③⑤⑥, the major band was detected at pI5.2 or lower, and smear was noted around pI6.5. In sample ②, the major band was detected around pI6.7. In control ⑦, not shown in the figure, the major band was detected around pI7.4. In contrast, in the patient sample, the major band was detected around pI6.5.

Table 1. Measurement of patient IgM by other machines.

Dilution	Cobas8000	H-7180	BM 6050	*BM-8030	C 16000	AU 640
× 1	1.85 g/L	2.69 g/L	1.13 g/L	1.01 g/L	4.79 g/L	6.61 g/L
× 10	3.95 g/L	3.81 g/L	4.22 g/L	3.67 g/L	3.73 g/L	3.83 g/L

Reagent: W company, * - N company.

Table 2. Analysis of combination ability of γ-globulin by BIACORE.

		Serum		Purified sample	
	Sample No.	IgM (g/L)	γ - G (RU)	IgM (g/L)	γ - G (RU)
Control	①	16.36	31	1.37	63
	④	18.73	3	1.37	80
	⑤	28.17	4	6.48	2
	⑥	43.00	46	8.69	100
	⑦	40.00	27	9.32	91
	⑧	12.53	36	4.64	93
Pt		22.90	72	6.76	107

Sample ②, ③ - not test.
RU - binding capacity.

A band was also detected at a point of pI5.2 or lower and it was not characteristic compared with that in the control (Figure 5).

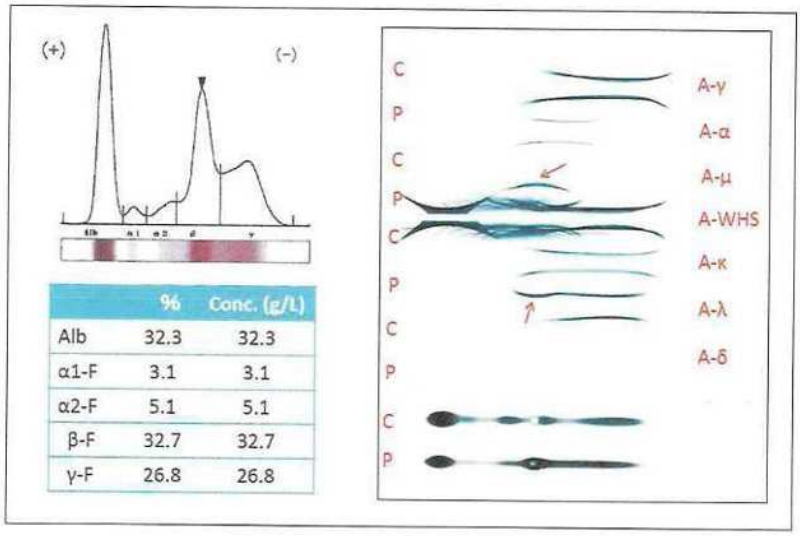
Hydrophobic interaction chromatography findings
The elution time was similar to that of control IgM. In addition, bimodal peaks were detected in contrast to single-peak M protein detected in many controls (Figure 6). Albumin-removed samples were also measured, but no difference from the control was noted.

Analysis of binding to γ-globulin using BIACORE
The γ-globulin binding was 4 - 46 RU in the control samples and 71 - 72 RU in the patient sample. It was 2 - 100 RU in the control samples after ammonium sulfate treatment, whereas it was 105 - 110 RU in the patient sample. Strong γ-globulin-binding ability was observed in the patient sample without ammonium sulfate treatment (Table 2).

DISCUSSION

In this study we reported IgM-type M protein although the true IgM level was high, no error code was displayed on the measurement devices when the undiluted sample was measured by TIA and it was measured as a low level. We considered it equipment failure at the beginning, but when measurement was performed using 6 models of 5 manufacturers used in Japan other than the model used in our laboratory, the IgM concentration was measured as a false low level by all devices. Therefore, we considered that it may be due to a specific physicochemical property of IgM of the patient with the false low level. Thus, using sera of 7 patients with IgM-type M protein as a control, we investigated the immunochemical and physicochemical characteristics of the patient IgM and discussed the developmental mechanism of the abnormal reaction.

In this study, the following experiments were performed to investigate the characteristics of the patient IgM: First, involvement of IgG in the abnormal reaction was confirmed because the IgG level was also abnormally high. To confirm it, we used 7 control samples of patients with IgM-type M protein and the patient sample in which IgM was decreased by treatment and resulted in getting an error code, the influence of addition of IgG was investigated. In the control samples, an error code was displayed even though the IgG level was high, but an error code was not displayed again in the patient sample, confirming that the patient IgM had a characteristic different from that of the other control IgM-type M proteins and the abnormal reaction occurred due to the involvement of IgG in the patient IgM. Since the ammonium sulfate fraction recovery rate was lower than that of the control samples in the purification process of



	%	Conc. (g/L)
Alb	32.3	32.3
α1-F	3.1	3.1
α2-F	5.1	5.1
β-F	32.7	32.7
γ-F	26.8	26.8

Figure 1-A. Protein fraction pattern of the patient serum.

M protein detected in the β fraction area (M protein: 32.7 g/L).

Figure 1-B. Immunoelectrophoresis pattern of the patient serum.

C - control serum, P - patient serum, A-γ - anti-human γ-heavy chain antibodies, A-α - anti-human α-heavy chain antibodies, A-μ - anti-human μ-heavy chain antibodies, A-WHS - anti human whole serum antibodies, A-κ - anti-human κ-light chain antibodies, A-λ - anti-human λ-light chain antibodies, A-δ - anti-human δ-heavy chain antibodies-
IgM λ-type M protein detected at the β area (indicated by arrows).

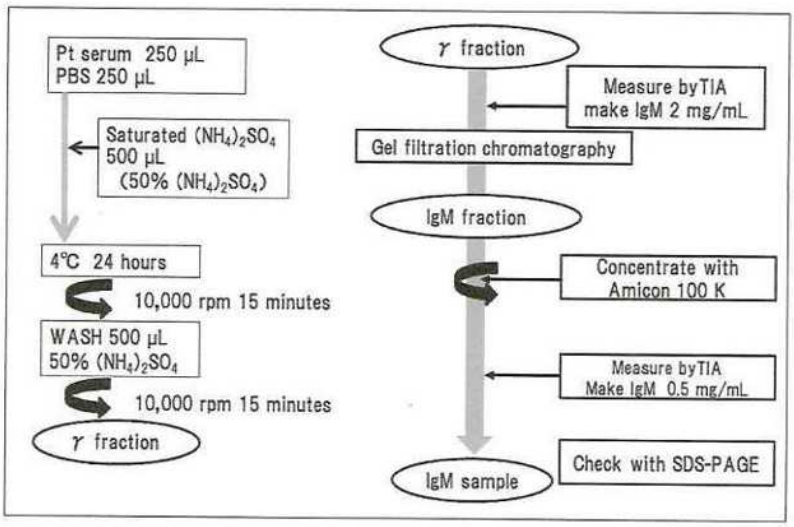


Figure 2. Purified of patient's IgM.

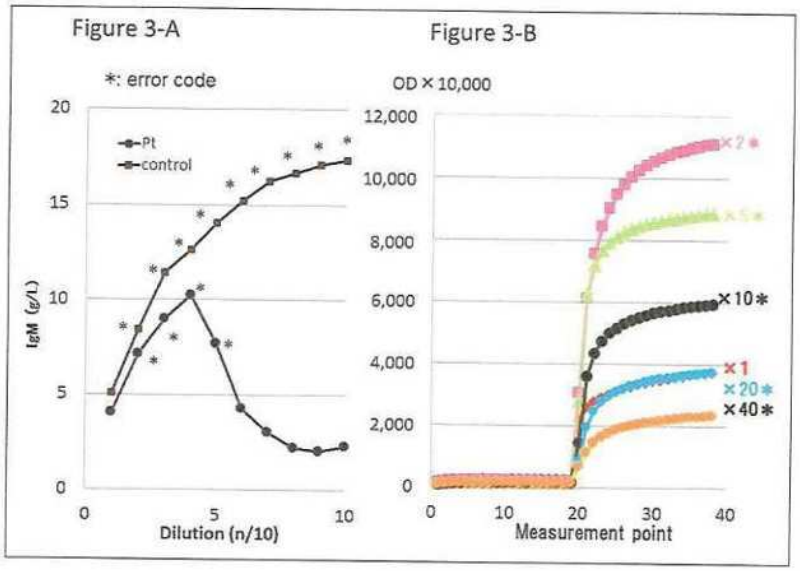


Figure 3-A. A straight course of dilution and error code.

The control sample produced an error code at all tested dilutions. In the patient sample (Pt), presumed IgM concentrations of 20.0 g/L or less did not trigger an error code.

Figure 3-B. Reaction time course of the patient IgM in Cobas 8000.

Over the first 20 points, the absorbance remained low for all samples. After the absorbance rose by more than twice the dilution, the sample triggered an error code.

the patient IgM, we presumed the presence of a difference in hydrophobicity between the patient IgM and control IgM. However, no difference was noted in the time-point of IgM detection on hydrophobic interaction chromatography, showing no difference in hydrophobicity. In addition, no difference was noted in the isoelectric point between the patient IgM and control IgM. Furthermore, in analysis using BIACORE, the KD (M) value was 1.08E-0.8 in the patient and 3.62E-0.8 in the control, showing no problem in the reactivity between the reagent (anti-IgM antibody) and sample (purified patient IgM). However, when γ-globulin was immobilized to BIACORE sensor chip to observe binding to IgG and the patient and control samples were applied and compared, significant binding to γ-globulin was observed in the patient sample. This binding was marked in the non-purified patient sample, suggesting that immune complexes formed by the patient IgM and surrounding plasma protein is involved in the abnormal reaction. Based on these findings, the abnormal reaction was unique to the patient IgM. It was suggested that the patient IgM strongly binds to IgG (non-covalent bond) and the non-covalently bound IgM-IgG complex inhibited reaction with anti-IgM antibody, which is the 2nd reaction reagent, in measurement of the original serum, leading to the false low level.

We previously encountered terminal liver cancer patients in whom IgG formed a complex with other plasma proteins in AFP measurement employing LBA-EATA. The complex inhibited the normal reaction system and led to a false low level, being similar to the developmental mechanism in the present patient. These abnormalities were improved by modifying the reaction matrix. In studies reporting an influence of other immunocomplexes on the measured value, IgA-type M protein bound to albumin and fructosamine was measured as a false high level [13], IgG-type M protein bound to anticoagulant, EDTA, and peripheral blood WBC was measured as a false high level on measurement using the mechanical method [14]. In addition, it has been frequently reported that a high level inconsistent with clinical findings discovered by isozyme test was due to immunoglobulin binding (anomaly) [15,16]. Furthermore, 1 year after encountering the present patient, we encountered a patient with other IgM-type M protein in whom no error code was displayed when the undiluted

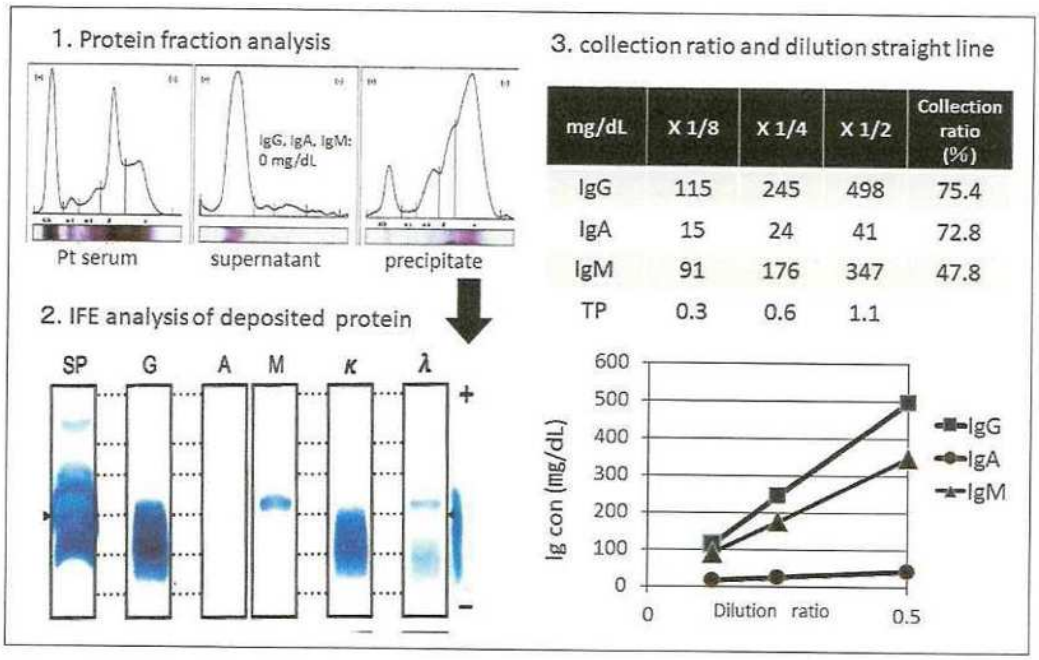


Figure 4. Analysis of treatment with ammonium sulfate.

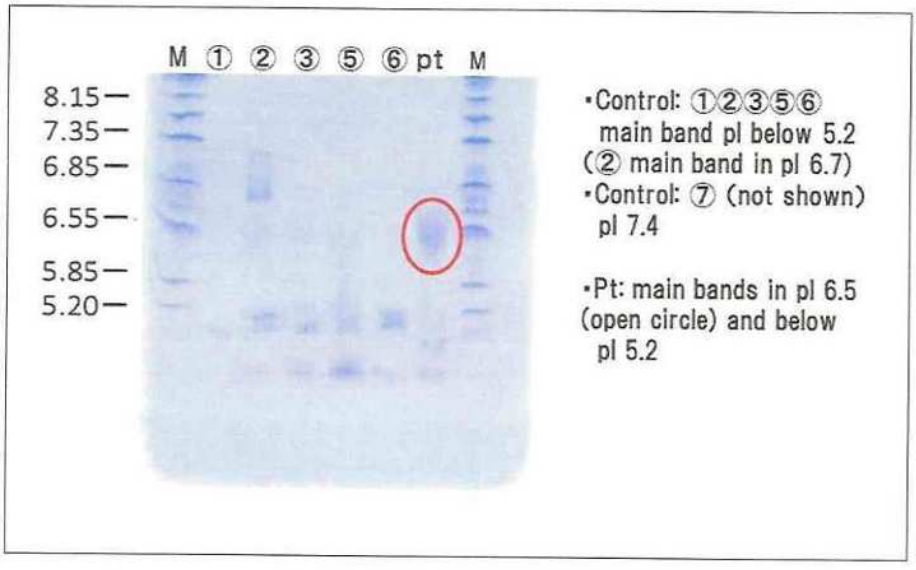


Figure 5. Patient's purified IgM pattern of isoelectric focusing.

A band was also detected at a point of pI5.2 or lower and it was not characteristic compared with that in the control.

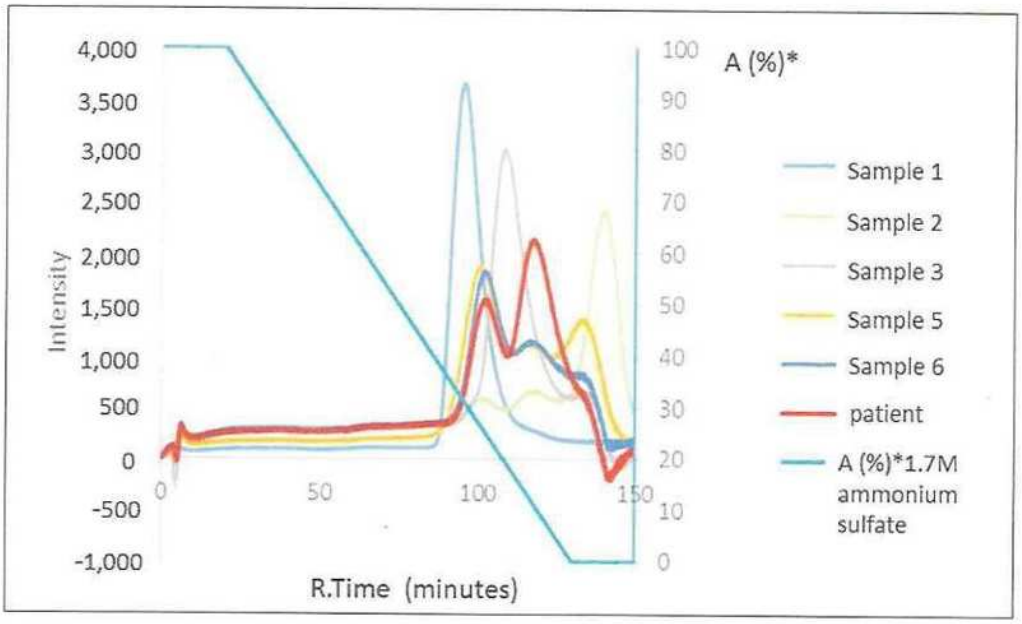


Figure 6. The elution time was similar to that of control IgM. Patient's purified IgM pattern of the hydrophobic chromatography.

sample was measured, as observed in the present patient, and IgM–κ-type M protein was measured as a false low level. On analysis of this 2nd case showing a false low-level IgM, white turbidity (also confirmed visually) occurred and this was presumed to be a reaction with PEG in the primary reaction. Even though a secondary antibody was added, the change in the absorbance was small and IgM was measured as a low level due to the influence of the turbidity of the primary reaction. Since the cause of the present case was the influence on PEG in the primary reaction, the values of IgA and IgM measured using the same reagent were also influenced. In the 2nd case, the reaction curve was markedly abnormal and IgA was measured as an abnormally low level depending on the measurement model, so that discovery of the abnormality may have been possibly depended on the measurement model. In the present study, the IgM concentration was also measured by nephelometry (measurement device: BN ProSpec, reagent 'N-anti-serum IgM'), but it was not measured as a false low level. This may have been due to the presence of the 'serial dilution' step in the nephelometry-based measurement system, which is not present in the TIA-based measurement systems generally used now.

A reaction process approximate analysis tool has recently been developed and enabled displaying an alert for erroneous measurement due to an unknown M protein, through which measurers can discover abnormality [17, 18]. However, in the present patient, the shape of the reaction curve was not abnormal and only a difference in the influence of dilution was observed, so that its detection may be difficult even though MiRuDa and analysis tools of JEOL and Beckman are used.

CONCLUSION

In this study, the immunochemical and physicochemical characteristics of IgM-type M protein measured as a false low level in immunoglobulin measurement by TIA were investigated. The patient M protein measured as a false low level had a high IgG binding ability, confirming its involvement in the occurrence of the abnormal reaction. We have already encountered 2 patients with different developmental mechanisms, suggesting the presence of other cases measured as a false low level.

Declaration of Interest:
The authors declare no conflict of interest.

References:

1. Bock JL, Furgiuele J, Wenz B. False positive immunometric assays caused by anti-immunoglobulin antibodies: a case report. Clin Chim Acta 1985;147(3):241-6. (PMID: 3888454)
2. Thompson RJ, Jackson AP, Langlois N. Circulating antibodies to mouse monoclonal immunoglobulins in normal subjects -incidence, species specificity, and effects on a two-site assay for creatine kinase-MB isoenzyme. Clin Chem 1986;32(3):476-81. (PMID: 3485019)
3. Müller W, Mierau R, Wohltmann D. Interference of IgM rheumatoid factor with nephelometric C-reactive protein determinations. J Immunol Methods 1985;80(1):77-90. (PMID: 3925019)
4. Montagna MP, Laghi F, Cremona G, Zuppi C, Barbaresi G, Castellana ML. Influence of serum proteins on fructosamine concentration in multiple myeloma. Clin Chim Acta 1991;31:123-30. (PMID: 1819455)
5. Kuramoto N, Wakahara T, Tamagawa Y, et al. A case of monoclonal gammopathy of undetermined significance with abnormal low levels of plasma glycated albumin by M protein. Clin Chim Acta 2018;487:337-40. (PMID: 30317021)
6. Kitaaki S, Hayashi N, Sato I, Watanabe K, Saegusa J, Kawano S. [Factors affecting non-specific reaction in latex agglutination turbidimetric immunoassay for detection of matrix metalloproteinase-3]. Rinsho Byori 2015;63(4):427-34. (PMID: 26536775)
7. Berth M, Delanghe J. Protein precipitation as a possible important pitfall in the clinical chemistry analysis of blood samples containing monoclonal immunoglobulins: 2 case reports and a review of the literature. Acta Clin Belg 2004;59(5):263-73. (PMID:15641396)
8. T Ohtake, S Kano, K Watanabe. [Interference in turbidimetric immunoassay for serum C-reactive protein due to serum protein abnormalities an immune complex and rheumatoid factor]. Rinsho Byori 2000;48(8):752-9. (PMID: 11064600)
9. Denham E, Mohn B, Tucker L, Lun A, Cleave P, Boswell DR. Evaluation of immunoturbidimetric specific protein methods using the Architect ci8200: comparison with immunonephelometry. Ann Clin Biochem 2007;44(6):529-36. (PMID: 17961307)
10. Kameko M, Kitamura H, Kawano M, Sakurabayashi I. [Evaluation of samples that indicated the difference of immunoglobulin values between different assays]. Rinsho Byori 2006;54(7):679-85. (PMID: 16913657)
11. Kubota N. [Immunoglobulin Quantitation by Latex Agglutination Immunoassay -New Sample Treatment to Reduce Differences in Measured Values by Turbidimetric Immunoassay]. Rinsho Byori 2010;58:387-92. (PMID: 20496767)
12. Thuillier F, Demarquilly C, Szymanowicz A, et al. [Nephelometry or turbidimetry for the determination of albumin, ApoA, CRP, haptoglobin, IgM and transthyretin: which choice?]. Ann Biol Clin (Paris) 2008;66(1):63-78. (PMID: 18227006)
13. Fujita K, Curtiss LK, Sakurabayashi I, et al. Identification and properties of glycated monoclonal IgA that affect the fructosamine assay. Clin Chem 2003;49:805-8. (PMID: 12709374)
14. Shimasaki AK, Fujita K, Fujio S, Sakurabayashi I. Pseudoleukocytosis without pseudothrombocytopenia induced by the interaction of EDTA and IgG2-Kappa M protein. Clin Chim Acta 2000; 299:119-28. (PMID: 10900298)
15. Levitt MD, Cooperband SR. Hyperamylasemia from the binding of serum amylase by an 11-S IgA globulin. N Engl J Med 1968: 278;474-9. (PMID: 4170008)
16. Fujita K. Immunochemical study of immunoglobulins bound to lactate dehydrogenase. Clin Chim Acta 1997:264;163-76. (PMID: 9293375)
17. Seimiya M, Suzuki Y, Yoshida T, Sawabe Y, Matsushita K, Nomura F. The abnormal reaction data-detecting function of the automated biochemical analyzer was useful to prevent erroneous total-bilirubin measurement and to identify monoclonal proteins. Clin Chim Acta 2015;441:44-6. (PMID: 25523191)
18. García-González E, Aramendía M, González-Tarancón R, Romero-Sánchez N, Rello L. Detecting paraprotein interference on a direct bilirubin assay by reviewing the photometric reaction data. Clin Chem Lab Med 2017;55(8):1178-85. (PMID: 28076302)