American Journal of Hematology 75:34–39 (2004)

A Non-immunological Phospholipid-Dependent Coagulation Inhibitor Associated With IgGλ-Type Multiple Myeloma

Osamu Takamiya,[1]* Shinichiro Machida,[2] Masahiro Okuda,[3] Junzo Nojima,[4] Chikako Koreeda,[5] and Katuhiko Kubara[6]

[1] Laboratory of Hematology, Department of Biomedical Laboratory Sciences, School of Health Sciences, Shinshu University, Matsumoto Asahi, Japan
[2] Division of Clinical Laboratory, Iki Public Hospital, Iki Gonoura, Japan
[3] Coagulation Group, Product Development Division, Sysmex, Murotani Kobe, Japan
[4] Laboratory of Hematology, Osaka University Hospital, Suita Osaka, Japan
[5] Internal Medicine, Iki Public Hospital, Iki Gonoura, Japan
[6] Kubara Clinic, Iki Noura, Japan

We investigated the rare case of a patient with IgGλ multiple myeloma for whom both prothrombin time and APTT were significantly prolonged. The IgG inhibited coagulation reactions upstream from prothrombin when coagulation was initiated by mRVVT, but not by FXa, as indicated by a chromogenic substrate for FXa. The mPT and the mAPTT showed inhibition of FXa generation in both the intrinsic and extrinsic pathways. The IgG inhibited both protein C (indicated by APTT) and FX (indicated by RVV) but not amidolysis for either activated protein C or FXa. The addition of excess phospholipid significantly shortened the prolonged RVVT of the patient. It inhibited the coagulation reactions of normal plasma and was dependent on decreasing the PS concentration in the APTT reagent. It was suggested that the IgG showed lupus anticoagulant (LA)-like activity that inhibited phospholipid-dependent coagulation reactions in the intrinsic, extrinsic, and common pathways. However, the IgG did not bind cardiolipin–β2GPI complex, β2GPI, or prothrombin in ELISA assays. The IgG did not bind to either PS or phospholipid complexes in the presence or absence of prothrombin, FX, or FXa. Interestingly, the IgG lost its LA like-activity when it was degraded to F(ab′)2 and Fc fragments by pepsin. We suspected that the IgG might inhibit the interaction between coagulation factors and acid phospholipid non-immunologically and that this process requires an intact IgG conformation, although the reaction mode is still not clear. Am. J. Hematol. 75:34–39, 2004. © 2003 Wiley-Liss, Inc.

Key words: multiple myeloma; IgGλ; phospholipid-dependent; non-immunological coagulation inhibitor; APTT

INTRODUCTION

It has been recognized that various hemostatic abnormalities are occasionally detected in patients with monoclonal gammopathies [1]. Patients with multiple myeloma frequently have abnormal coagulation tests, including thrombin times (64%), fibrin degradation products (32%), platelet aggregation tests carried out with different agonists (30–55%), and bleeding times (22%) [2]. The abnormal coagulation tests are owing to the paraprotein from patients with multiple myeloma, which also can inhibit von Willebrand factor [3], act as circulating anticoagulants [4,5], and disrupt fibrin monomer polymerization [6]. The incidence of bleeding complications is low,

*Correspondence to: Dr. Osamu Takamiya, Laboratory of Hematology, Department of Biomedical Laboratory Sciences, School of Health Sciences, Shinshu University, Matsumoto Asahi 390-8621, Japan. E-mail: itosamu@gipac.shinshu-u.ac.jp

Received for publication 20 January 2003; Accepted 15 August 2003

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ajh.10449

despite the diversity of abnormal haemostatic tests observed for these patients [7]. Clinically significant bleeding had been described in approximately 15% of patients with IgG myeloma, 40% of patients with IgA myeloma, and up to 60% of patients with IgM myeloma or Waldenström's macroglobinemia [8]. Massive hemorrhage was determined to be the cause of death in 3% of patients with multiple myeloma [9]. Massive hemorrhage has been reported following transbronchial biopsy and cytoscopy. Hemorrhagic problems should always be considered in the decision to perform these procedures on a patient with multiple myeloma [8]. On the other hand, thrombotic complications have been reported to occur in approximately 25% of multiple myeloma patients with the lupus anticoagulant [9].

We describe herein a λ-type IgG from a patient with myeloma, which was found during a coagulation screening test prior to colonoscopy. The patient's IgG inhibited phospholipid-dependent coagulation reactions, and its LA-like activity disappeared, when it was degraded to F(ab′)2 and Fc fragments by pepsin.

CASE REPORT

A 57-year-old woman was referred to Iki Public Hospital on February 12, 2002, to have a colonoscopy performed to evaluate bleeding she experienced during defecation. The patient had been on follow-up for the chemotherapy and hypertension at a neighboring clinic, after diagnosis of λ-type IgG multiple myeloma at a hospital. Complete blood count showed a hemoglobin level of 9.8 g/dl, a platelet count of $122 \times 10^3/\mu l$, and a white blood cell count (WBC) of $2.3 \times 10^3/\mu l$. Marked rouleaux formation was noted on the peripheral smear, and a WBC differential count identified 1.5% atypical lymphocytes. A bone marrow examination revealed 32% plasma cells, which was consistent with multiple myeloma. The levels of immunoglobulin showed marked monoclonal increases: IgG, 3,310 mg/dl; IgA, 24 mg/dl; IgM, 17 mg/dl. No Bence-Jones protein (BJP) was found in the patient's urine. Roentgenograms identified numerous small "punch-out" osteolytic lesions in her skull. Table I summarizes the results of her coagulation profile. Both PT and APTT were markedly prolonged, and she had a slightly high fibrinogen concentration and a normal FDP. Mixing of her plasma with an equal amount of normal plasma did not correct either the prolonged PT or the APTT. Pro-coagulant activities of her coagulation factors were within normal reference limits except factor XIIc. The patient's plasma was diluted from 20-fold to 40-fold into normal plasma to avoid the influence of possible circulating inhibitors, because an equal mixing with normal plasma did not correct prolonged PT and APTT. Actin (Dade, Miami, FL), which was not sensitive for LA [10], was used for measurement of coagulation factors FVIII, FIX, FXI, and FXII. The presence of an LA was suggested by both diluted APTT and RVVT tests. The phospholipids-neutralizing LA test (Gradipore, Frenchs Forest, Australia) was positive. RVVT and APTT were carried out after mixing the patient's plasma with normal pooled plasma, and these showed a typical convex curve (not shown). These results suggested the presence of lupus anticoagulant. The patient's clinician recognized later that the bleeding at the time of defecation was due to hemorrhoids. She now has neither bleeding nor a thrombotic tendency.

TABLE I. Results of Coagulation Test

Bleeding time	2 min 30 sec
Prothrombin time (11.0 sec)	19.1 sec
APTT (31.5 sec)	128.6 sec
Thrombotest	30.2%
Fibrinogen	
Clotting assay	440 mg/dl
Immunoturbidity assay	490 mg/dl
FDP	< 5 μg/ml
Factor II	118%
Factor V	100%
Factor VII	136%
Factor VIII	92%
Factor IX	165%
Factor X	104%
Factor XI	81%
Factor XII	45%
Lupus anticoagulant	(+)
LA test (Gradipore) (< 1.3)	1.68

Isolation of IgG From Patient's Plasma

Citrate anticoagulated blood was centrifuged twice at 3,000 rpm for 10 min to obtain platelet-free plasma, and the plasma was stored at −80°C. The patient's IgG was purified by a protein G Sepharose 4 Fast Flow column (Pharmacia Biotech, Uppsala, Sweden). The purified IgG was immediately dialyzed into TBS (150 mM NaCl, 50 mM Tris-HCl, pH 7.4). The concentration of purified IgG was determined from A_{280}, using an extinction coefficient of $1.35\ mg^{-1}\ ml\ cm^{-1}$.

Preparation of F(ab′)2, Fab′, and Fc Fragments

F(ab′)2, Fab′, and Fc fragments were prepared by a method described previously [11]. Briefly, the IgG fraction after dialysis against 0.1 M acetate buffer (pH 4.5) was digested with 0.4% pepsin overnight at 37°C. After adjustment to pH 8.0 with 1 N NaOH, the reaction mixture was applied to a Sephadex G-150 column (1.5 × 40 cm) previously equilibrated with 0.1 M sodium borate buffer (pH 8.0), and eluted with the same buffer to yield the F(ab′)2 fraction. A

portion of this fraction was dialyzed against 0.1 M acetate buffer, reduced with 10 mM 2-mercaptoethylamine for 90 min at 37°C and passed through a Sephadex G-25 column (1.0 × 30 cm) with the same buffer to separate the Fab′ fragment.

Detection of LA Activity

LA activity was assayed by diluted APTT and diluted RVVT to screen for LA [12]. RVVT and APTT mixing studies were carried out by mixing the patient's plasma with normal pooled plasma (5:0, 4:1, 1:1, 1:4, and 0:5). The phospholipids-neutralizing test (Gradipore) was then carried out for confirmation.

Modified PT and Modified APTT

Modified PT (mPT) and modified APTT (mAPTT) were designed to detect coagulation reactions upstream from FX. Five microliters of the patient's IgG (3 mg/ml) or normal IgG (3 mg/ml) was incubated with 10 μl of normal plasma. After 5 min at 37°C, mPT reagent or mAPTT reagent was added, and release of *p*-nitroaniline was measured by recording the absorbance at 504 nm continuously for 5 min. The rate of change in the absorbance at 1-min intervals was assayed. The mPT reagent consisted of 50 μl of 3 mM S-2772 (Chromogenix, Molndal, Sweden), 25 μl of 500 mM $CaCl_2$, 325 ml of Tris buffer (tris-hydroxymethylmethane; 50 mmol/l, NaCl 227 mmol/l, pH 8.3), and 100 μl of PT reagent (Inovin, Deerfield, IL). The mAPTT was used as the APTT reagent in place of the PT reagent.

Modified RVVT

A modified RVVT (mRVTT) was designed to measure common pathway upstream from prothrombin in the coagulation system. Five microliters of the patient's IgG (3 mg/ml) or normal IgG (3 mg/ml) was incubated with 10 μl of normal plasma. After 5 min at 37°C, mRVVT reagent was added, and release of *p*-nitroaniline was measured by recording the absorbance at 504 nm continuously for 5 min. The rate of change in the absorbance at 1-min interval was assayed. The mRVVT reagent consisted of 50 μl of 3 mM S-2238 (Chromogenix, Molndal, Sweden), 25 μl of 500 mM $CaCl_2$, 325 ml of Tris buffer, and 100 μl of RVV (Sigma, St. Louis, MO).

Effect of IgG on Phospholipid-Independent Assay for FX

For a phospholipid-independent FX activity assay, 5 μl of patient IgG (3 mg/ml) or normal IgG (3 mg/ml) was incubated with 10 μl of purified human FXa (Haematologic Technologies, Inc., Essex Junction, VT). After 5 min at 37°C, S-2772 was added. The release of *p*-nitroaniline measured by recording the absorbance at 504 nm after 5 min.

Effect of IgG on Phospholipid-Dependent and Phospholipid-Independent Assay for Protein C

For a phospholipid-dependent protein C activity assay, 5 μl of the patient's IgG (3 mg/ml) or normal IgG (3 mg/ml) was incubated with 50 μl of normal plasma. After being incubated for 5 min at 37°C, the plasma mixture and 50 μl of Protac C (American Diagnostica, Greenwich, CT) were added to 50 μl of protein C depleted plasma. After being incubated for 1 min at 37°C, 50 μl of APTT reagent (actin, Dade) was added to the second mixture. Fibrin formation was measured with Amelung KC4A micro, after adding 50 μl of $CaCl_2$ (25 mM) following incubation for 1 min at 37°C.

For a phospholipid-independent protein C activity assay, 5 μl of the patient's IgG (3 mg/ml) or normal IgG (3 mg/ml) was incubated with 10 μl of activated protein C, which was activated in normal plasma by Protac C. After 5 min at 37°C, chromogenic substrate for protein C (Immuno, Vienna, Heidelberg, Germany) was added. The release of *p*-nitroaniline was measured by recording the absorbance at 504 nm after 5 min.

Effect of Phospholipid Composition of APTT on the Patient's IgG

Three APTT reagents containing different phosphatidylserine concentrations were prepared according to a method described previously [13,14].

L-Phosphatidylethanolamine (PE), L-phosphatidylcholine (PC), L-phosphatidylserine (PS) (Avanti Polar Lipids, Alabaster, AL), butylated hydroxyanisole (BHA; Nakari, Osaka, Japan), cholesterol (Nakarai), and chloroform were mixed in a glass container. The mixture was dried under reduced-pressure nitrogen at room temperature. APTT reagent was prepared and suspended in 50 mM HEPES-Tris buffer (pH 7.35) containing 0.35% phenol and ellagic acid (0.10 mmol/l) by sonication. Phospholipid in APTT–PSM reagent was composed of PE (30 μg/ml), PC (60 μg/ml), and PS (20 μg/ml). APTT–PSM reagent was composed of PE (30 μg/ml), PC (60 μg/ml), and PS (20 μg/ml). APTT–PSL reagent was composed of PE (30 μg/ml), PC (60 μg/ml), and PS (5 μg/ml). The samples, which included normal plasma added to either patient's IgG or to normal IgG, were assayed using these three APTT reagents.

ELISA for Anti-Prothrombin, Anti-β2GPI, and Anti-Cardiolipin

Anti-cardiolipin–β2GP I complex was detected by ELISA using a kit purchased from Yamasa (Yamasa Shoyu, Chosi, Japan).

Both anti-prothrombin and anti-β2GP I were detected using a specific ELISA system that contained human prothrombin or β2GP I that were directly immobilized on γ-irradiated polystyrene ELISA plate (Nunc-immunoplate, MaxiSorp, Kamstrup, Roskilde, Denmark) [15].

ELISA for Binding IgG to PS, PE, and Phospholipid Complex

Microplate wells were coated with 100 μl of PS (50 μg/ml) or PE (50 μg/ml) in ethanol or 100 μl of phospholipid complex, which was prepared by the method mentioned above, but without ellagic acid. The wells were blocked with TBS containing 0.3% gelatin and 1% BSA and left overnight at 4°C. To measure the binding IgG to phospholipid in the presence of prothrombin, factor X, or factor Xa, the phospholipid-coated microplate wells were coated with 100 μl of prothrombin (50 μg/ml), factor X (50 μg/ml), or factor Xa (50 μg/ml) in TBS buffer with 5 mmol/l Ca^{2+}. One hundred microliters of test sample (50 μg/ml patient IgG or normal IgG) was added to each well, and any IgG that bound to the wells was then detected by incubating the wells with anti-human IgG labeled with peroxidase (Dako, Kyoto, Japan). The resulting immunocomplexes were revealed using H_2O_2 and *o*-phenylenediamine.

RESULTS

In order to clarify the cause of both the prolonged PT and the prolonged APTT, the effect of the patient's IgG on common pathways of the coagulation system was assayed. Although the patient's IgG (f.c., 2 mg/ml) did not inhibit the thrombin time of purified fibrinogen, the thrombin generation of FIX-deficient plasma assay by mRVVT was inhibited relative to assays carried out with normal IgG. The effect of the inhibition was dose-dependent in a range from 0.5 to 5.0 mg/ml of the patient's IgG. Next, the effect of the patient's IgG on FXa generation by mPT and mAPTT was assayed in order to detect the effect of the patient's IgG on FXa generation in the intrinsic or extrinsic coagulation pathways. The patient's IgG significantly inhibited FXa generation in comparison with normal IgG in both mPT and mAPTT assays. Finally, FX and protein C activities were assayed using both phospholipid-dependent and phospholipid-independent coagulation tests. The amidolytic activity of FXa was not affected in the presence of the patient's IgG. Protein C activity, which was assayed by APTT using protein C-depleted plasma as a substrate, increased in the presence of the patient's IgG. The prolonged APTT seen in the protein C assay resulted from increasing protein C activity. The amidolytic activity of Protac-activated protein C, however, was not affected in the presence of the patient's IgG. Table II summarizes the results mentioned above.

The mixture of normal plasma plus normal IgG did not show significant differences in clotting times among the APTT–PSH reagent (PS: 50 μg/ml), the APTT–PSM reagent (PS: 20 μg/ml), and the APTT–PSL reagent (PS: 5 μg/ml). However, the clotting time of the mixture of normal plasma plus the patient's IgG was prolonged, in increasing order, as follows: APTT–PSL reagent > APTT–PSM reagent > APTT–PSH reagent. The patient's IgG inhibited coagulation reactions that depend on the PS concentration of the APTT reagent.

The patient's serum was negative for both IgG and IgM (anti-prothrombin) and was negative for both IgG and IgM (anti-β2GP) according to assays using irradiated polystyrene ELISA plates. The serum was also revealed to be negative for anti-cardiolipin–β2GPI complex IgG. The binding ability of patient's IgG to immobilized PS, to immobilized PE and to immobilized phospholipid complex was examined in an ELISA. The binding ability of the patient's IgG to PS, PE, and phospholipid complex did not exceed that of normal IgG used as a control. Furthermore, the binding ability of the patient's IgG to PS, PE, and phospholipid complex in the presence of prothrombin, FX, and FXa also did not exceed that of normal IgG used as a control (Table III).

In order to measure the effect of F(ab′)2 and Fc fragments on coagulation reactions by Thrombocheck APTT–SLA (Sysmex Co., Kobe, Japan), 10 μl each of the F(ab′)2 fraction (5 mg/ml) or the Fc fraction (5 mg/ml) was prepared from the patient's

TABLE II. Effect of IgG

Method	Patient's IgG	Normal IgG
Thrombin time[a]	18.4 sec	20.3 sec
mRVVT[b]	0.0288	0.0621
mPT[b]	0.0187	0.0216
mAPTT[b]	0.0355	0.0754
FXa (chromgenic assay)[c]	0.610	0.608
Protein C (clotting assay)[a]	230%	100%
Protein C (chromgenic assay)[c]	0.504	0.486

[a]Clotting assay.
[b]Rate assay (A_{504}: O.D.).
[c]Endpoint assay (A_{504}: O.D.).

TABLE III. Binding Ability of IgG to Phospholipid in the Presence and Absence of Prothrombin, Factor X, and Factor Xa*

	Patient's IgG				Normal IgG			
	Absence	+FII	+FX	+FXa	Absence	+FII	+FX	+FXa
PS	0.038	0.041	0.038	0.040	0.032	0.033	0.041	0.036
PE	0.037	0.049	0.039	0.043	0.031	0.050	0.038	0.038
PL complex	0.026	0.016	0.017	0.021	0.026	0.018	0.016	0.022

*A_{504}: O.D.

IgG or from normal IgG added to 50 μl of normal plasma. Although the patient's IgG showed a remarkable prolonged APTT (110.6 sec) in comparison to buffer (42.0 sec) as a control, neither the patient's F(ab′)2 (44.0 sec) or Fc (41.8 sec) inhibited the APTT of normal plasma. Normal IgG (42.2 sec), F(ab′)2 (42.8 sec), and Fc (41.8 sec) had the same effect on coagulation time as did the buffer control.

DISCUSSION

In the present study, we investigated a rare case of patient with IgGλ multiple myeloma for whom both prothrombin time and APTT were significantly prolonged. Robert et al. reported that a prolonged thrombin time is the most common abnormal coagulation parameter found in patients with IgG multiple myeloma; the prothrombin time was prolonged in 11% of patients with IgG myeloma, but none of the patients had a prolonged APTT [2]. Sixteen percent of patients with light-chain disease had an abnormal APTT, but none of these patients had a prolonged prothrombin time [2]. Saif et al. reported a patient with multiple myeloma showing both a prolonged thrombin time and a prolonged prothrombin time. Addition of this patient's IgGλ corrected the prothrombin time in mixing studies and showed a near-correction of thrombin time, but no correction of replitase time [16].

Neither the prolonged prothrombin time nor the prolonged APTT of our patient was corrected by mixing with normal plasma. The patient's IgG did not inhibit the thrombin time of purified fibrinogen. The coagulation factors were within normal ranges except for FXII, which might have been affected by the inhibitor present in the plasma. The IgG of our patient inhibited coagulation reactions in the common pathway upstream from prothrombin that were initiated by mRVVT, but not FXa by mRVVT using a chromogenic substrate for FXa. The mPT and the mAPTT indicated inhibition of FXa generation in both the intrinsic and extrinsic pathways. Furthermore, the patient's IgG inhibited phospholipid-dependent coagulation reactions of normal plasma. This was indicated by a protein C assay initiated by APTT and using protein C depleted plasma, and by a FX assay initiated by RVV. However, amidolysis for both activated protein C and FXa was not affected.

The addition of excess phospholipid significantly shortened the prolonged RVVT of the patient. It inhibited the coagulation reactions of normal plasma that depend on the PS concentration in the APTT reagent. It was suggested that the IgG of our patient inhibited phospholipid-dependent coagulation reaction in the intrinsic, extrinsic, and common pathways. However, we could not detect the binding of cardiolipin–β2GPI complex, β2GPI, or prothrombin to the patient's antibody by ELISA assays. The IgG did not bind to PS in the presence or absence of prothrombin, FX, or FXa, or to phospholipid complexes in the presence or absence of prothrombin, FX, or FXa. The epitope for most of LA consists of a complex of anionic phospholipid and plasma protein such as β2GPI and prothrombin [17]. Bevers et al. explained that LA were not directed to phospholipids alone, but presumably recognized an epitope that became exposed upon Ca^{2+}-mediated binding of human prothrombin to phospholipids [18]. Yasin et al. reported that κ light chains from the urine of a patient with multiple myeloma possessed LA and they reasoned that this may represent a hydrophobic interaction with phospholipids, because the antibody demonstrated no significant phospholipid-binding activity in ELISA assay using dried lipids [5]. Sakakura et al. reported that 25% of the patients who were positive for LA were positive for anti-phospholipid antibodies, and that 17% were positive for an anti-cardiolipin–β2GPI complex antibody [10].

The IgG of our patient might inhibit phospholipid-dependent coagulation reaction as LA-like activity. Interestingly, addition of the patient's IgG removed LA-like activity, even when it was degraded to F(ab′)2 and Fc fragments by pepsin. Thiagarajan et al. reported that the Fabμ tryptic fragments from IγMλ of a patient with macroglobulinemia inhibited the phospholipid-dependent coagulation reactions

[19]. However, Bellotti et al. reported that the F(ab′)2 from IgGκ multiple myeloma had lost anticoagulant activity, and suggested that an electrostatic interaction with negatively charged phospholipids was responsible for the anticoagulant activity [20]. Shinagawa et al. reported that the dimeric λ-type BJP associated with multiple myeloma showed LA-like activity, which may be dependent on the ionic interaction between Ig and anionic phospholipid rather than constituting specific antigen–antibody interactions [4]. Non-immunological interactions of either the light chains or IgG F(ab′)2 have been reported [21]. Fujita described that BJP (or IgG), which possesses a conformation similar to NAD binding site, combined non-immunologically with lactate dehydrogenase [22].

In summary, we suspect that the IgG of our patient with multiple myeloma might inhibit the interaction between coagulation factors and acid phospholipid non-immunologically, thus affecting coagulation reactions while maintaining an intact IgG conformation, although the exact reaction mode is not yet clear.

REFERENCES

1. Furie B. Acquired coagulation disorders and dysproteinemias. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, editors. Hemostasis and thrombosis. Philadelphia: J.B. Lippincott Company; 1987. p 577–581.
2. Robert F, Mignucci M, McCurcy SA, Maldonado N, Lee JY. Hemostatic abnormalities associated with monoclonal gammopathies. Am J Med Sci 1993;306:359–366.
3. Michiels JJ, Budde U, van der Planken M, van Vliet HH, Schroyens W, Berneman Z. Acquired von Willebrand syndromes: clinical features, aetiology, pathophysiology, classification and management. Best Pract Res Clin Haematol 2001;14:401–436.
4. Shinagawa A, Kojima H, Kobayashi T, Kawada K, Nagasawa T. Lupus anticoagulant-like activity observed in a dimeric lambda protein produced by myeloma cells. Int J Hematol 2001;73: 526–531.
5. Yasin Z, Quick D, Thiagarajan P, Spoor D, Caraveo J, Palascak J. Light-chain paraproteins with lupus anticoagulant activity. Am J Hematol 1999;62:99–102.
6. O'Kane MJ, Wisdom GB, Desai ZR, Archbold GP. Inhibition of fibrin monomer polymerisation by myeloma immunoglobulin. J Clin Pathol 1994;47:266–268.
7. Aoki S, Sibata A. Anemia and hemostatic abnormalities associated with mulitiple myeloma. Nippon Rinsho 1995;53:154–158 (in Japanese).
8. Glaspy JA. Hemostatic abnormalities in multiple myeloma and related disorders. Hematol Oncol Clin North Am 1992;6: 1301–1314.
9. Kapadia SB. Multiple myeloma: a clinicopathologic study of 62 consecutively autopsied cases. Medicine (Baltimore) 1980;59: 380–392.
10. Sakakura M, Wada H, Watanabe R, Mamamuro M, Okugawa Y, Nakasaki T, et al. Coagulation tests and anti-phospholipid antibodies in patients positive for lupus anticoagulant. Clin Appl Thromb Hemost 2000;6:144–150.
11. Takamiya O. Studies on protein C. II. Biological and immunological assay of human protein C. J Nara Med Assoc 1984;35: 448–459.
12. Alving BM, Barr CF, Johansen LE, Tang DB. Comparison between a one-point dilute phospholipid APTT and the dilute Russell viper venom time for verification of lupus anticoagulants. Thromb Haemost 1992;67:672–678.
13. van den Besselaar AM, Neuteboom J, Bertina RM. Related articles. Effect of synthetic phospholipids on the response of the activated partial thromboplastin time to heparin. Blood Coagul Fibrinolysis 1993;4:895–903.
14. Bock PE, Srinivasan KR, Shore JD. Related articles. Activation of intrinsic blood coagulation by ellagic acid: insoluble ellagic acid–metal ion complexes are the activating species. Biochemistry 1981;20:7258–7266.
15. Nojima J, Kitani T, Iwatani Y, Kanakura Y. Anti-prothrombin antibodies combined with lupus anticoagulant activity is an essential risk factor for venous thromboembolism in patients with systemic lupus erythematosus. Br J Haematol 2001;114:647–654.
16. Saif MW, Allegra CJ, Greenberg B. Bleeding diathesis in multiple myeloma. J Hematother Stem Cell Res 2001;10:657–660.
17. Shapiro SS. The lupus anticoagulant/antiphospholipid syndrome. Annu Rev Med 1996;47:533–553.
18. Bevers EM, Galli M, Barbui T, Comfurius P, Zwaal RF. Lupus anticoagulant IgG's (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. Thromb Haemost 1991;66:629–632.
19. Thiagarajan P, Shapiro SS, De Marco L. Monoclonal immunoglobulin M lambda coagulation inhibitor with phospholipid specificity. Mechanism of a lupus anticoagulant. J Clin Invest 1980;66:397–405.
20. Bellotti V, Gamba G, Merlini G, Montani N, Bucciarelli E, Stoppini M, et al. Study of three patients with monoclonal gammopathies and "lupus-like" anticoagulants. Br J Haematol 1989;73:221–227.
21. Erntell M, Myhre EB, Kronvall G. Non-immune IgG F(ab′)2 binding to group C and G streptococci is mediated by structures on gamma chains. Scand J Immunol 1985;21:151–157.
22. Fujita K. Immunochemical study of immunoglobulins bound to lactate dehydrogenase. Clin Chem Acta 1997;264:163–176.