

[ORIGINAL ARTICLE]

Elevation of Plasmin- α 2-plasmin Inhibitor Complex Predicts the Diagnosis of Systemic AL Amyloidosis in Patients with Monoclonal Protein

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Abstract:

Objective The complication of systemic immunoglobulin light chain (AL) amyloidosis in patients with monoclonal immunoglobulin affects the prognosis, but amyloid deposition in tissues is sometimes difficult to detect due to bleeding tendencies and preferential distributions. However, fibrinolysis is known to be exacerbated in patients with systemic AL amyloidosis specifically. We therefore explored new biomarkers for predicting a diagnosis of systemic AL amyloidosis focusing on coagulation and fibrinolysis markers.

Methods We reviewed the clinical features and treatment outcomes of patients with serum monoclonal protein, including primary systemic AL amyloidosis and multiple myeloma (MM), treated at our hospital between January 2008 and December 2014.

Results Among several biomarkers, only the serum level of plasmin- α 2-plasmin inhibitor complex (PIC) in patients with systemic AL amyloidosis (n=26) at the diagnosis was significantly higher than in patients with MM without AL amyloidosis (n=26) (mean \pm standard deviation, 3.69 \pm 2.82 μ g/mL vs. 1.23 \pm 0.97 μ g/mL, p < 0.01). The cut-off for predicting a diagnosis of systemic AL amyloidosis in patients with serum monoclonal protein was 1.72 μ g/mL with 84.6% sensitivity and 80.8% specificity. Hepatic involvement resulted in a significantly higher PIC level than no involvement in patients with systemic AL amyloidosis. The serum PIC level was also associated with the hematological response of systemic AL amyloidosis.

Conclusion PIC is a useful biomarker for the diagnosis and management of patients with systemic AL amyloidosis.

Key words: systemic immunoglobulin light chain (AL) amyloidosis, multiple myeloma (MM), plasmin- α 2-plasmin inhibitor complexes (PIC)

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Introduction

Systemic immunoglobulin light chain (AL) amyloidosis is a rare disorder caused by the deposition of insoluble amyloid fibrils, which comprise fragments of monoclonal light chains produced by abnormal plasma cells. This deposition occurs in the heart, kidneys, gastrointestinal tract, liver, lungs, nerve, and soft tissues, resulting in organ dysfunction (1). AL deposition arises as a complication of multiple myeloma (MM) at a frequency of 15-30% (2). In patients

presenting with serum and/or urinary monoclonal protein, systemic AL amyloidosis must be distinguished from MM because AL deposition, particularly in the heart, shortens the survival (1).

A biopsy of the involved organs is required to diagnose AL amyloidosis. However, a biopsy of organs such as the heart, kidney, and liver is problematic and carries a high risk of bleeding in patients with systemic AL amyloidosis (3). Abdominal fat pad aspirate for the diagnosis is a less invasive procedure, and its sensitivity is 60-80%; however, the remaining cases still need a biopsy of the involved or-

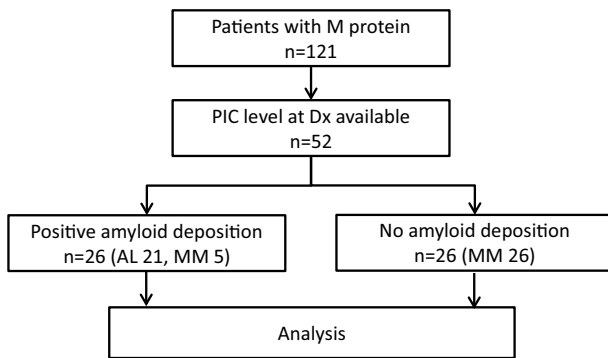


Figure 1. The analysis profile. AL: primary systemic immunoglobulin light chain amyloidosis, MM: multiple myeloma, PIC: plasmin- α 2-plasmin inhibitor complex, Dx: diagnosis

gans (4, 5). Repeated biopsies are often needed in some situations. A new biomarker for predicting a diagnosis of systemic AL amyloidosis is therefore greatly needed.

Fibrinolysis is known to be exacerbated in patients with systemic AL amyloidosis (3, 6-10). A few studies have reported elevated levels of plasmin- α 2-plasmin inhibitor complex (PIC) in patients with systemic amyloidosis (3, 6, 8, 10). However, whether or not the elevation of PIC is specific to patients with systemic AL amyloidosis or common in patients with serum monoclonal protein is unclear. In this study, we present the clinical features and treatment outcomes of patients with serum monoclonal protein, with a particular focus on coagulation and fibrinolysis markers, in order to show the significance of PIC in the diagnosis of AL amyloidosis.

Materials and Methods

Patients

We reviewed the medical charts of 121 unselected, consecutive patients with serum and/or urine monoclonal protein seen at Sapporo Medical University Hospital between January 2008 and December 2014. Of these, 52 patients for whom PIC data were available from before treatment were analyzed in this study. We excluded patients with thrombosis, aortic aneurysm, diabetes mellitus, or antithrombotic drugs, as those factors might influence the serum PIC levels. The diagnosis of AL amyloidosis and organ involvement was based on the consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis (11) and the definition of organ involvement and response to treatment in AL Amyloidosis: an Updated Consensus Opinion (12). This study was approved by the institutional review board of Sapporo Medical University Hospital.

Analyses of coagulation and fibrinolysis markers

PIC was measured in all patients before treatment. The prothrombin time international normalized ratio (PT-INR), activated partial thromboplastin time (APTT), fibrinogen, fi-

brinogen degradation product (FDP), D-dimer and thrombin-antithrombin complex (TAT) were used for analysis if available. PIC, FDP, and D-dimer were measured by latex agglutination turbidimetry. The PT-INR and APTT were measured by the coagulation method. Fibrinogen was measured by the thrombin method. TAT was measured by a chemiluminescence immunoassay. The reference ranges were based on the criteria at our institution.

Treatments and response evaluation

Patients received various treatments as chosen by the attending physician, including melphalan plus dexamethasone, bortezomib-containing regimens, high-dose melphalan, and autologous stem cell transplantation (HD-MEL/ASCT), as the first-line therapy. The hematological response was evaluated according to the consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis (11), the definition of organ involvement and response to treatment in AL Amyloidosis: an Updated Consensus Opinion (12), and the validation of the criteria for response to treatment in AL amyloidosis (13).

Statistical analyses

Differences were compared between two groups using the *t*-test or the Wilcoxon signed-rank test. The $p < 0.05$ indicates a significant difference. A receiver operating characteristic (ROC) curve was drawn to decide a cut-off value with optimum sensitivity and specificity. All statistical analyses were conducted using the EZR software program on R Commander, version 1.32 (freely available on the website <http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html>).

Results

Patient characteristics

Among 121 patients with serum and/or urine monoclonal protein, we retrospectively analyzed the 52 for whom PIC levels were available at the diagnosis. The deposition of amyloid was pathologically confirmed in at least 1 organ in 26 patients, comprising 21 patients with primary systemic AL amyloidosis and 5 with MM (secondary systemic AL amyloidosis). The remaining 26 patients with no amyloid deposition were diagnosed with MM (Fig. 1).

In patients with primary and secondary systemic AL amyloidosis, the median age at diagnosis was 63.0 years (range, 37-75 years), and 15 (58%) were men. The type of monoclonal protein was light chain-only in 13 patients, IgG in 6, IgA in 4, and IgD in 3. The light chain was kappa in 3 patients and lambda in 23. The median amount of monoclonal protein of IgG, IgA and IgD was 1,810 mg/dL (range, 1,142-3,615 mg/dL), 1,546 mg/dL (range, 411-2,197 mg/dL), and 199 mg/dL (range, 55.3-328 mg/dL), respectively. The median percentage of bone marrow plasma cells was 3.7% (range, 0.7-80%) in patients with systemic AL amyloidosis. However, it was 3.2% (range, 0.7-8.8%) in patients

Table 1. Patient Characteristics.

Patient Variable	systemic AL amyloidosis n (%) or median (range)	MM without AL amyloidosis n (%) or median (range)
Age, y	63 (37-75)	68 (34-84)
Gender, male	15 (58)	10 (38)
Type of M protein (heavy chain)		
Light chain only		
IgG	13 (50)	6 (23)
IgA	6 (23)	15 (58)
IgD	4 (15)	4 (15)
IgD	3 (12)	1 (4)
Type of M protein (light chain)		
Kappa	3 (12)	12 (46)
Lambda	23 (88)	14 (54)
Amount of M protein (mg/dL)		
IgG	1,810 (1,142-3,615)	4,830 (1,523-9,878)
IgA	1,546 (411-2,197)	1,035 (605-2,824)
IgD	199 (55.3-328)	2,900
Percentage of BMPC	3.7 (0.7-80)	40.8 (10.2-90)
Number of organs involved		
1	6 (23)	
2	8 (31)	
3	7 (27)	
4	3 (12)	
5	2 (7)	
Organ involvement		
Heart	15 (58)	
Kidney	18 (69)	
Gastrointestinal tract	18 (69)	
Liver	4 (15)	
Peripheral nervous system	1 (4)	
Soft tissue	8 (31)	
Lung	1 (4)	
First-line therapy		
Bortezomib-containing	22 (85)	
High-dose melphalan+ASCT	1 (4)	
Melphalan+dexamethasone	2 (7)	
Others	1 (4)	

systemic AL amyloidosis: systemic immunoglobulin light chain amyloidosis, MM: multiple myeloma, M protein: monoclonal protein, BMPC: bone marrow plasma cells, ASCT: autologous stem cell transplantation

with primary systemic AL amyloidosis (without MM) and 29.8% (range, 12.6-80%) in patients with secondary systemic AL amyloidosis (with MM). The median number of organs involved was 2 (range, 1-5), with involvement of the heart in 58%, kidney in 69%, gastrointestinal tract in 69%, and liver in 15%. As first-line therapy, 22 patients (85%) received bortezomib-containing regimens.

In the 26 patients without amyloid deposition, the median age at the diagnosis was 68.0 years (range, 34-84 years), and 10 (38%) were men. The type of monoclonal protein was light chain-only in six patients, IgG in 15, IgA in four, and IgD in one. The light chain was kappa in 12 patients and lambda in 14. The median amount of monoclonal protein of IgG, IgA and IgD was 4,830 mg/dL (range, 1,523-9,878 mg/dL), 1,035 mg/dL (range, 605-2,824 mg/dL), and 2,900 mg/dL, respectively. The median percentage of bone

marrow plasma cells was 40.8% (range, 10.2-90%). The patient characteristics are summarized in Table 1.

A comparison of the coagulation and fibrinolysis markers between patients with and without amyloidosis

We first analyzed the differences in coagulation and fibrinolysis markers between patients with and without AL amyloidosis (Table 2). In both groups, the mean values for PT-INR, APTT and TAT were within the institutional reference ranges, but the mean values for PIC, FDP, and D-dimer exceeded the upper limits of normal. Among these, only the serum PIC level showed a significant difference, with patients with AL amyloidosis showing significantly higher serum PIC levels than those without AL amyloidosis [mean± standard deviation (SD), 3.69±2.82 µg/mL vs. 1.23±0.97 µg/

Table 2. Comparison of Coagulation and Fibrinolysis Markers between Patients with and without Amyloidosis.

Patient Variable	(reference range)	systemic AL amyloidosis mean±SD (n)	MM without AL amyloidosis mean±SD (n)	p value
PIC (µg/mL)	(0-0.8)	3.69±2.82 (26)	1.23±0.97 (26)	<0.01
PT-INR	(0.85-1.14)	1.07±0.11 (26)	1.13±0.15 (26)	0.10
APTT (s)	(18-36)	29.48±5.27 (26)	30.38±3.88 (26)	0.48
Fibrinogen (mg/mL)	(213-332)	378±95.3 (20)	319±144.4 (13)	0.16
FDP (µg/mL)	(0-5)	8.35±4.76 (18)	7.40±6.36 (24)	0.60
D-dimer (µg/mL)	(0-1)	2.92±2.98 (17)	2.90±3.10 (18)	0.99
TAT (ng/mL)	(0-3)	2.98±1.31 (18)	2.82±2.05 (19)	0.78

systemic AL amyloidosis: systemic immunoglobulin light chain amyloidosis, MM: multiple myeloma, PIC: plasmin- α -2-plasmin inhibitor complexes, PT-INR: prothrombin time-international normalized ratio, APTT: activated partial thromboplastin time, FDP: fibrinogen degradation product, TAT: thrombin-antithrombin complex

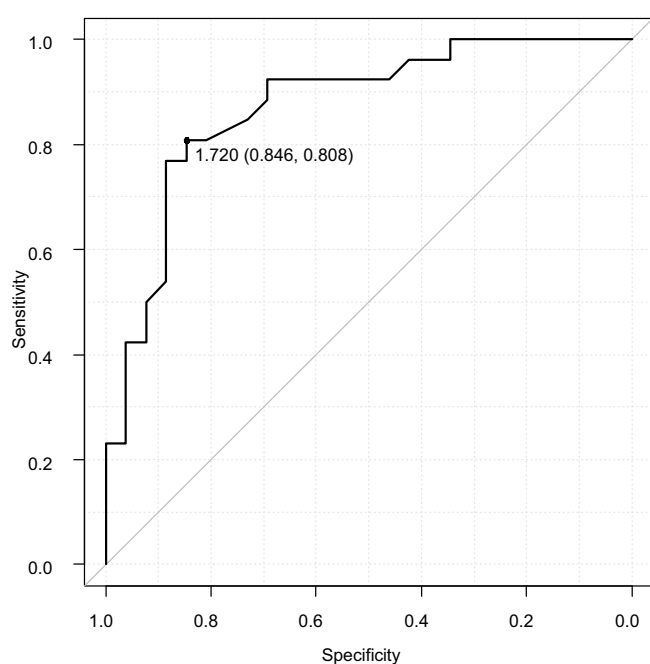


Figure 2. Cut-off value for predicting systemic AL amyloidosis. The cut-off value for predicting a diagnosis of systemic AL amyloidosis in patients with serum monoclonal protein was 1.72 µg/mL, offering 84.6% sensitivity and 80.8% specificity.

mL, $p < 0.01$, t -test]. No marked difference in the serum PIC level was seen between patients with primary ($n=21$) and secondary ($n=5$) systemic AL amyloidosis (mean±SD, 4.06 ± 2.99 µg/mL vs. 2.12 ± 1.04 µg/mL, $p=0.13$, t -test).

We next drew the ROC curve to determine the optimum cutoff value for using the serum PIC level to predict a diagnosis of AL amyloidosis in patients with serum monoclonal protein (Fig. 2). The optimum value of 1.72 µg/mL offered 84.6% sensitivity and 80.8% specificity.

Relationship between the serum PIC level and organ involvement or hematological response in patients with systemic AL amyloidosis

We next analyzed the relationship between the serum PIC level and organ involvement in patients with systemic AL

amyloidosis (Table 3). Hepatic AL patients ($n=4$) showed significantly higher serum PIC levels than patients without hepatic involvement ($n=22$) (mean±SD, 7.25 ± 3.25 µg/mL vs. 3.04 ± 2.26 µg/mL, $p=0.01$, t -test). No other organ depositions were associated with significant increases in the serum PIC level.

We finally analyzed the association between changes in serum PIC level and hematological responses. We compared the serum PIC levels before treatment and at the best hematological response in 23 patients with AL amyloidosis. Serum PIC levels were significantly decreased in patients with complete response (CR) ($n=14$) (mean±SD, 3.66 ± 3.02 µg/mL vs. 2.16 ± 1.27 µg/mL, $p < 0.01$, Wilcoxon signed-rank test). No significant difference was evident in patients with non-CR ($n=9$) (mean±SD, 3.46 ± 2.00 µg/mL vs. 3.05 ± 2.38 µg/mL, $p=0.20$, Wilcoxon signed-rank test). The changes in the serum PIC levels in patients with hematological CR are shown in Fig. 3.

Discussion

The present study revealed that the serum PIC levels in patients with systemic AL amyloidosis were significantly higher than those in patients without amyloidosis. This observation seems likely to help in the diagnosis of systemic AL amyloidosis in patients with serum and/or urine monoclonal protein. The optimum cut-off value for the serum PIC level for predicting systemic AL amyloidosis was 1.72 µg/mL, with 84.6% sensitivity and 80.8% specificity.

Systemic AL patients have a tendency to bleed, due to the activation of fibrinolysis (3, 6-10). A few previous studies have reported elevated serum PIC levels in patients with systemic amyloidosis (3, 6, 8, 10), although the underlying mechanisms have yet to be elucidated. Several hypotheses for the elevation of PIC have been proposed, such as the production of urokinase-type plasminogen activator (uPA) from bone marrow plasma cells in AL amyloidosis (6), stimulation of the expression of uPA and its receptor by amyloid beta-protein in human cerebrovascular smooth muscle cells (14), enhanced tissue-type plasminogen activator

Table 3. Relationship between Serum PIC Level and Organ Involvement.

	Organ involvement		p value
	Positive mean±SD (n)	Negative mean±SD (n)	
Heart	4.06±2.74 (15)	3.18±2.98 (11)	0.20
Kidney	4.16±3.21 (18)	2.62±1.20 (8)	0.31
Gastrointestinal tract	3.72±2.68 (18)	3.61±3.30 (8)	0.97
Liver	7.25±3.25 (4)	3.04±2.26 (22)	0.01
Peripheral nervous system	1.6 (1)	3.77±2.84 (25)	-
Soft tissue	3.33±2.91 (8)	3.84±2.85 (18)	0.55
Lung	2.6 (1)	3.73±2.87 (25)	-

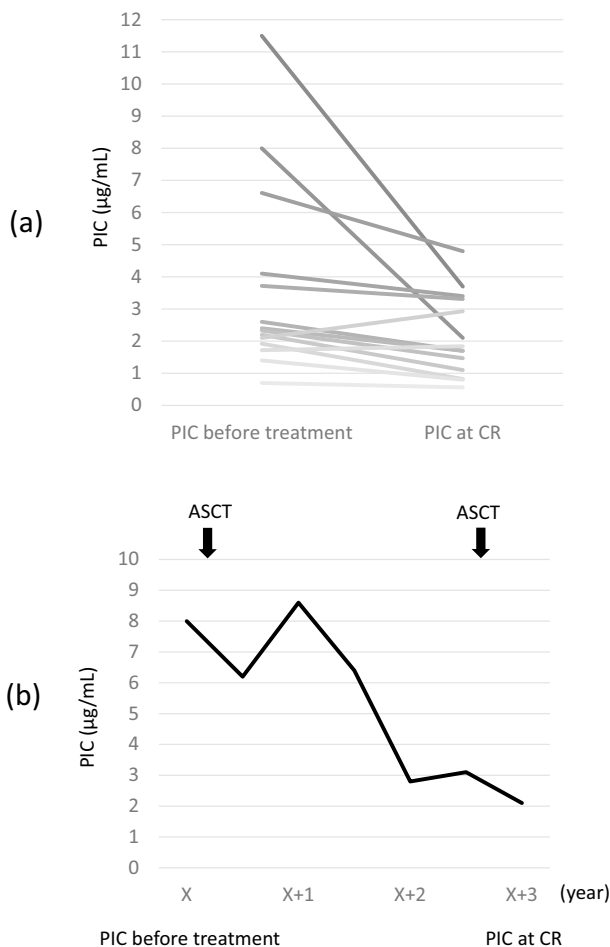


Figure 3. Changes in the serum PIC levels in patients with hematological CR. (a) Changes in the serum PIC levels before treatment and at hematological CR (n=14). (b) Changes in the serum PIC levels in the patient with the second-highest PIC level before treatment among total patients. PIC: plasmin- α 2-plasmin inhibitor complex, CR: complete response, ASCT: autologous stem cell transplantation

(tPA) activity associated with binding of tPA to multiple cross-beta-folded peptides including amyloid (7, 8, 15), and activation of plasminogen caused by amyloid proteins directly via tPA or indirectly via the kinin-kallikrein system (16). Our observations confirm and advance the findings of previous reports indicating high serum PIC concentrations

in AL patients.

The mean values of PIC, FDP, and D-dimer exceeded the reference ranges in both patients with and without AL deposition. In contrast, the mean values for PT-INR, APTT and TAT were within the reference ranges in both groups. Venous thromboembolic events are more common in patients with MM due to multiple factors, including inflammatory cytokines, such as interleukin (IL)-6 and vascular endothelial growth factor (VEGF) (7). All patients enrolled in our analysis showed no obvious thrombosis before treatment. However, the above mechanisms might have influenced values of PIC, FDP, and D-dimer in both groups of patients. However, only the serum PIC levels were significantly higher in patients with AL amyloidosis than in those without.

We analyzed the relationship between the serum PIC levels and organ involvement at the diagnosis in systemic AL patients. Hepatic AL patients showed significantly higher serum PIC levels than patients with other organ involvement. The liver produces plasmin and α 2-plasmin inhibitor, so PIC may be generated directly in the liver in the presence of amyloid protein. Since a liver biopsy has the potential to result in bleeding complications in a small percentage of AL patients, liver involvement is determined by non-specific findings, such as hepatomegaly and the elevation of alkaline phosphatase levels. Our results may be helpful for the non-invasive diagnosis of hepatic AL amyloidosis.

We also analyzed the association between changes in the serum PIC level and the hematological response in systemic AL patients. The serum PIC level after CR was significantly decreased compared with before treatment. This observation indicates that serum PIC levels are associated with the hematological response in systemic AL amyloidosis.

Although some reports have suggested the elevation of PIC levels in patients with AL amyloidosis (3, 6, 8, 10), this is the first to show the importance of serum PIC levels in both the diagnosis of AL amyloidosis and the evaluation of treatment responses among patients with AL amyloidosis. However, the limitations of our study include its retrospective study design, the single-institutional setting, and the small population size. Accordingly, a prospective trial needs to be performed to confirm our findings in the future.

In conclusion, PIC is a useful biomarker for the diagnosis

and management of patients with systemic AL amyloidosis.

The authors state that they have no Conflict of Interest (COI).

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