Immune complexome analysis of serum samples from non-small-cell lung cancer patients identifies predictive biomarkers for nivolumab therapy

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Abbreviations: **AUC**, area under the curve; **IC**, immune complex; **ICI**, immune checkpoint inhibitor; **nano-LC-MS/MS**, nano-liquid chromatography-tandem mass spectrometry; **MSI-H**, microsatellite instability-high; **NSCLC**, Non-small-cell lung cancer; **PD-1**, programmed cell death 1, **PD-L1**, programmed death-ligand 1; **PFN1**, Prorfilin-1; **ROC**, receiver operating characteristics: **TAA**, tumor-associated antigen; **TMB**, Tumor mutation burden

Keywords: immune complex antigen; immune complexome analysis; non-small-cell lung cancer; nivolumab; therapeutic predictive biomarker

Abstract

Background: Immune checkpoint inhibitors (ICIs) have achieved important outcomes in cancer treatment. However, current clinical biomarker tests are not suitable for some patients because they require tumor tissues and have poor predictive value for treatment responses. Therefore, the identification of biomarkers that enable screening tests in all patients is necessary.

Methods: We performed an immune complexome analysis of non-small cell lung cancer patients treated with nivolumab to comprehensively identify and compare antigens incorporated into immune complexes (IC-antigens) in serum samples from the responders (n = 15) and non-responders (n = 20). Additionally, combinations of IC-antigens characteristic to the responder group were evaluated by logistic regression analysis and receiver operating characteristics curves to examine their predictiveness for ICI treatment responses.

Results: The combination of predictive biomarkers detected before treatment was profilin-1, purine nucleoside phosphorylase, alpha-enolase, and nucleoside diphosphate kinase A [p = 0.0043, odds ratio = 2.26, 95% confidence interval (CI) = 1.19–4.28, area under the curve = 0.76]. The combination of predictive biomarkers detected after treatment was peptidyl-prolyl cis-trans isomerase A, ubiquitin-like modifier-activating enzyme 1, complement component C8 beta chain, and apolipoprotein L1 (p = 0.0039, odds ratio = 2.56, 95% CI = 1.25–5.23, area under the curve = 0.77).

Conclusion: Combinations of serum IC-antigens may predict the therapeutic effect of nivolumab in non-small cell lung cancer patients.

Introduction

Various immune checkpoint inhibitors (ICIs) have been developed. ICI therapy is included in advanced neutrophil-to-lymphocyte ratio (NSCLC) treatment guidelines [1], and these agents are indispensable for cancer therapy. Nivolumab is a fully humanized IgG4 antibody against programmed cell death 1 (PD-1) [2] that blocks the interaction between PD-1 expressed on activated T cells and its ligand programmed death-ligand 1 (PD-L1) on tumor cells. This blockade enhances immune responsiveness, leading to tumor elimination [3]. However, because of the limited predictive biomarkers for ICI treatment responses, these expensive agents are used for not only responders (20%–30% of patients receiving therapy) but also non-responders. Furthermore, ICIs are associated with immune-related adverse events, which may lead to death [4]. For these reasons, long-term administration to non-responders should be avoided, but it may take more than a few months after the initiation of treatment to confirm the effect based on clinical findings. Therefore, it is necessary to identify biomarkers for predicting therapeutic responses before administration or immediately after starting treatment. Distinguishing between responders and non-responders will facilitate individualized ICI treatment, decrease unnecessary treatment, and improve the cost-effectiveness of ICIs.

Immune complexes (ICs) are formed when antigens bind to antibodies [5] and are the direct and real-time products of immune responses [6]. To date, the detection of circulating ICs involving cancer antigen 125 in ovarian cancer patients [7] and IC detection in patients with chemotherapy-treated pancreatic cancer [8] have been proposed for cancer diagnosis or response predictions. We developed an immune

complexome analysis method to comprehensively identify antigens in ICs (IC-antigens) using IC-capturing beads and nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS). Using this method, we identified that gelsolin was specifically and frequently detected in ICs in patients with advanced NSCLC [9].

In this study, we aimed to discover IC-antigens with biomarker characteristics for advanced NSCLC patients responsive to nivolumab therapy by comprehensively comparing serum IC-antigens between responders and non-responders. Serum IC-antigens may provide non-invasive predictive biomarkers for responders, regardless of the cancer type and patient status. Therefore, we aimed to identify groups of IC-antigens preferentially detected in the responder group. Finally, we evaluated the groups by logistic regression and receiver operating characteristic (ROC) curve analyses.

Material and Methods

Serum samples were collected from 35 NSCLC patients treated with nivolumab. Patients were classified as responders (n = 15; age, 54–77 years; partial response or stable disease for more than 6 months) and non-responders (n = 20; age, 46–80 years) (Table 1). Patients with stage III, stage IV, or postoperative recurrent NSCLC who did not show a treatment response after completing their regimens (except ICIs) were enrolled in this study. Histological types were adenocarcinoma (n = 21), squamous cell (n = 9), large-cell neuroendocrine carcinoma (n = 2), undifferentiated (n = 1), both adenocarcinoma and squamous cell (n = 1), and both squamous cell and large-cell

neuroendocrine carcinoma (n = 1). Serum samples were collected from each patient before nivolumab administration and 1 or 2 weeks after the first dose. Sample collection and diagnosis were performed at Nagasaki University Hospital or Tochigi Cancer Center. All experiments were performed in accordance with the Helsinki Declaration and with approval from the institutional ethics committees of the Graduate School of Biomedical Sciences, Nagasaki University (approval number: 160725154) and Tochigi Cancer Center (approval number: A-374). Each patient provided written informed consent for their participation in this study.

ICs were purified using three types of IC-capturing beads (Protein G-coated magnetic beads [PureProteome®, Millipore, Darmstadt, Germany], Protein A-coated magnetic beads [PureProteome®], and Proceptor™-Sepharose beads [ProGen Biologics, Wildwood, MO, USA]). Each bead type (40 μL) was incubated with 10 μL of patient serum diluted in 90 μL phosphate-buffered saline (PBS) for 30 min at room temperature with gentle mixing, and then the liquid was removed. Further processing was conducted as described in our previous study [10]. In this experiment, we used papain, which digests antibodies at their hinge region, to selectively recover (elute) antigens and Fab fragments from ICs collected on magnetic beads. This procedure excludes non-specific binding proteins from the nano-LC-MSMS analysis for identifying antigens. All IC-antigens collected using the three types of beads were integrated. We compared IC-antigens between the responder group and the non-responder group.

Univariable logistic regression analysis was used to determine the value of age, sex, histological type, and sets of IC-antigens in predicting the nivolumab treatment

outcome. To evaluate the prediction accuracy of independent significant predictors, ROC curves and the resulting area under the curves (AUCs) were constructed. The optimal cutoff point was determined as the point at which the Youden index was maximized by the ROC curve. Statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. All statistical analyses were performed with JMP® 15 (SAS Institute Inc., Cary, NC, USA).

Results

We identified 1304 IC-antigens in serum samples collected from each patient before the administration of nivolumab using immune complexome analysis. The number of antigens identified by each bead type was as follows: Protein G-coated magnetic beads, 605; Protein A-coated magnetic beads, 594; and Proceptor™-Sepharose beads, 493. Comparing IC-antigens, five antigens were detected 25% more frequently in the responder group than in the non-responder group. Univariable logistic regression analysis showed that a set of four IC-antigens [profilin-1 (PFN1), purine nucleoside phosphorylase, alpha-enolase, and nucleoside diphosphate kinase A] (Table 2) referred to as the "first set" significantly predicted the effect of nivolumab treatment [$p = 0.0043$, odds ratio = 2.26 with 95% confidence interval (CI) = 1.19 to 4.28]. The other factors (age, sex, and histological type) were not significant. Subsequently, a ROC curve was generated for the first set (AUC = 0.76, Fig. 1A). The cutoff value was 2, with a sensitivity of 60% and specificity of 80% (Table 3).

We identified 1507 IC-antigens in serum samples collected after the

158 administration of nivolumab for the first time using immune complexome analysis. The
159 number of antigens identified by each bead type was as follows: Protein G-coated
160 magnetic beads, 712; Protein A-coated magnetic beads, 576; and
161 Proceptor™-Sepharose beads, 645. Among the IC-antigens, 12 antigens were detected
162 25% more often in the responder group than in the non-responder group. Univariable
163 logistic regression analysis showed that a combination of four IC-antigens
164 (peptidyl-prolyl cis-trans isomerase A, ubiquitin-like modifier-activating enzyme 1,
165 complement component C8 beta chain, and apolipoprotein L1) (Table 2) referred to as
166 the "second set" significantly predicted the effect of nivolumab treatment ($p = 0.0039$,
167 odds ratio = 2.56 with 95% CI = 1.25 to 5.23). Subsequently, a ROC curve was
168 generated for the second set (AUC = 0.77, Fig. 1B). The cutoff value was 2, with a
169 sensitivity of 80% and specificity of 75% (Table 3).
170
171 **Discussion**
172 Tumor PD-L1 expression determined by immunohistochemistry is used as a
173 predictive biomarker for the response to pembrolizumab in patients with advanced
174 NSCLC [11], but the use of PD-L1 as a predictor of ICI treatment appears to be limited.
175 On the other hand, tumor mutation burden (TMB) is considered a surrogate biomarker
176 of immunotherapy sensitivity because mutations in tumor cells are thought to produce
177 neoantigens that are targets of immune therapy [12-14]. Indeed, microsatellite
178 instability-high (MSI-H) tumors are now treated with ICIs. The amount of
179 tumor-infiltrating lymphocytes has also been reported as a predictive biomarker [15,16].

However, these biomarker tests are highly invasive and sometimes require biopsies [17] and tumor heterogeneity may make it difficult to evaluate the entire tumor [18]. Furthermore, although PD-L1 expression and TMB are considered biomarkers of ICI sensitivity, previous studies reported that neither were correlated to the responsiveness of advanced NSCLC patients to PD-1 blockade therapy [19, 20]. TMB and MSI status are related, and MSI-H patients may have a high TMB. The above reports support that it may be difficult and insufficient to predict prognosis and efficacy using only a single biomarker. Combining several biomarkers may improve the predictive therapeutic ability, indicating that multiple factors may be involved in the response to nivolumab.

IC-antigens are proteins targeted by the immune system following ICI administration. In patients who show a good response to ICI treatment, characteristic IC-antigens may be present because the response possibly reflects protein abnormalities targeted by the immune system. Increased abnormalities may lead to a better response through a stronger immune attack by ICI-activated immune cells [21, 22]. These abnormal proteins induce the production of antibodies that eventually form ICs in responders before ICI treatment and may also be released from tumor cells after ICI treatment. Our analysis noninvasively identified groups of the IC-antigens that were predictive for nivolumab responsiveness before and after treatment. IC-antigens identified using our method included tumor-associated antigens produced by several mechanisms, not limited to mutations (neoantigens). Different predictive IC-antigen sets were identified before and after treatment, possibly due to the occurrence of associated antigens released from dead cells in the presence or absence of therapy. Here, we found

an association between serum IC-antigens and immunotherapy outcomes in advanced NSCLC and showed that a combination of several IC-antigens predicted the therapeutic effect of nivolumab. Several groups have studied responder biomarkers using a minimally invasive approach, such as peripheral blood samples [23]. For example, a high neutrophil-to-lymphocyte ratio is associated with lower response rates to ICIs [24,25]. However, the search for additional minimally invasive biomarkers using peripheral blood remains insufficient. Regarding the prediction of clinical outcomes by the neutrophil-to-lymphocyte ratio, Jiang et al. reported a sensitivity of 87% and specificity of 46% [23], and Liu et al. reported a sensitivity of 81% and specificity of 73% [25]. In our study, the sensitivity and specificity were 60% and 80% for the first set and 80% and 75% for the second set, respectively, with a similar predictive ability as previous studies.

Considering the relationship between each identified protein and the therapeutic effect, PFN1 was found to be associated with the response to nivolumab, but the relationship between the seven identified proteins and treatment responsiveness cannot be clearly explained. PFN1 is a member of the actin-binding protein family. Additionally, PFN1 is reported to be the only member of the PFN family dominantly expressed in primary human CD8+ T cells and to be a negative regulator of the cytotoxic T lymphocyte-mediated elimination of target cells [26]. PFN1 overexpression in endothelial cells line may stabilize cell junctions, and PFN1 downregulation in lung adenocarcinoma cells suppresses cell migration and sensitizes the cells to anticancer agents [27]. PFN1 may be antigenic due to mutations or structural abnormalities caused

by disease, which impairs its ability to function as a negative regulator. Therefore, the administration of nivolumab reactivates T cells and cytotoxic T lymphocytes to target cancer cells. Furthermore, mutations in F-actin-binding proteins (FABPs), including PFN1, occur frequently in most human cancers and generate tumor neoantigens in both mice and humans [28,29]. Moreover, these mutated FABPs are cross-presented by type 1 conventional dendric cells to prime anticancer CD8+ T cells [30]. The cross-presentation occurs through lectin receptor DNGR-1 highly expressed on the cells [30], and gelsolin selectively impairs this process [30]. Because high levels of gelsolin are found in the circulation, alterations in gelsolin may occur. Using our method, we previously detected this protein as an IC-antigen [9]. Therefore, the increased detection of PFN1 in this study is consistent with previous studies.

This study showed that a combination of several IC-antigens in serum predicted the therapeutic effect of nivolumab. PD-L1 expression reflects only one of the signals in the immune system, and the prediction of treatment responses depends on this signal. Therefore, it is reasonable that PD-L1 expression and treatment outcomes are not correlated in some patients. TMB is the total amount of somatic mutations in a tumor, but mutations do not necessarily result in the generation of abnormal proteins. Even if the TMB is high, it does not always correspond to the high number of antigens targeted when the immune system is reactivated by ICIs. The immune response against a tumor is thought to be triggered by autologous proteins of tumor cells, commonly referred to as tumor-associated antigens, which may be mutated, misfolded, degraded, proteolytically cleaved, or overexpressed [31]. Furthermore, because there is a 20-fold

difference in mutation prevalence between human cancer types, unbiased screening analysis for neoantigens by DNA or RNA sequencing is essentially limited to tumor types with a large number of mutations, such as melanoma [32]. Therefore, identifying cancer-specific antigens should not be limited to neoantigens, and all tumor-associated antigens should be included in our screening [9]. In this context, immune complexome analysis detects tumor-associated antigens as IC-antigens and provides a promising tool to identify predictive biomarkers for diagnosis and treatment response and to develop therapeutic targets for cancer immunotherapy.

The limitations of this study include a small sample size, and it remains unclear whether these sets of IC-antigens apply to therapies with other ICIs. Our proposal should be validated using a larger sample size with enzyme-linked immunosorbent assays for high-throughput measurements. Additionally, IC-antigens characteristic of responders to other ICI treatments remain to be identified.

Conclusion

This study indicates that combinations of serum IC-antigens may predict the therapeutic effect of nivolumab in NSCLC patients. Immune complexome analysis may be used to screen biomarkers for responders to ICI therapy, and the use of these serum biomarkers provides a non-invasive approach that can be used in several patients. The measurement of IC-antigen biomarkers by enzyme-linked immunosorbent assays may be more useful to determine the response to ICIs.

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References

[1] N. H. Hanna *et al.*, “Therapy for stage IV non–small-cell lung cancer without driver alterations: ASCO and OH (CCO) joint guideline update,” *J. Clin. Oncol.*, vol. 38, no. 14, pp. 1608–1632, 2020.

[2] C. Wang *et al.*, “In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates,” *Cancer Immunol. Res.*, vol. 2, no. 9, pp. 846–856, 2014.

[3] J. R. Brahmer *et al.*, “Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: Safety, clinical activity, pharmacodynamics, and immunologic correlates,” *J. Clin. Oncol.*, vol. 28, no. 19, pp. 3167–3175, 2010.

[4] A. Winer, J. Nicholas Bodor, and H. Borghaei, “Identifying and managing the adverse effects of immune checkpoint blockade,” *J. Thorac. Dis.*, vol. 10, no. 9, pp. S480–S489, 2018.

[5] N. Aibara and K. Ohyama, *Revisiting immune complexes: Key to understanding immune-related diseases*, vol. 96. 2020.

[6] K. Ohyama *et al.*, “Immune Complexome Analysis of Serum and Its Application in Screening for Immune Complex Antigens in Rheumatoid Arthritis,” vol. 909, pp. 905–909, 2011.

[7] D. W. Cramer *et al.*, “CA125 immune complexes in ovarian cancer patients with low CA125 concentrations,” *Clin. Chem.*, vol. 56, no. 12, pp. 1889–1892, 2010.

[8] G. Mandili *et al.*, “Immune-Complexome Analysis Identifies Immunoglobulin-Bound Biomarkers That Predict the Response to Chemotherapy of Pancreatic Cancer Patients,” *Cancers (Basel).*, 2020.

[9] K. Ohyama *et al.*, “Immune complexome analysis reveals the specific and frequent presence of immune complex antigens in lung cancer patients: A pilot study,” *Int. J. Cancer*, vol. 140, no. 2, pp. 370–380, 2017.

[10] N. Aibara *et al.*, “Selective, sensitive and comprehensive detection of immune

complex antigens by immune complexome analysis with papain-digestion and elution," *J. Immunol. Methods*, vol. 461, no. June, pp. 85–90, 2018.

[11] A. Ribas *et al.*, "Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): A randomised, controlled, phase 2 trial," *Lancet Oncol.*, vol. 16, no. 8, pp. 908–918, 2015.

[12] A. M. Goodman *et al.*, "Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers," *Mol. Cancer Ther.*, vol. 16, no. 11, pp. 2598–2608, 2017.

[13] N. A. Rizvi *et al.*, "Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer," *Science (80-.).*, vol. 348, no. 6230, pp. 124–128, 2015.

[14] S. Champiat, C. Ferté, S. Lebel-Binay, A. Eggermont, and J. C. Soria, "Exomics and immunogenics: Bridging mutational load and immune checkpoints efficacy," *Oncoimmunology*, vol. 3, no. 1, 2014.

[15] P. C. Tumeh *et al.*, "PD-1 blockade induces responses by inhibiting adaptive immune resistance," *Nature*, vol. 515, no. 7528, pp. 568–571, 2014.

[16] S. Kumagai *et al.*, "The PD-1 expression balance between effector and regulatory T cells predicts the clinical efficacy of PD-1 blockade therapies," *Nat. Immunol.*, vol. 21, no. 11, pp. 1346–1358, 2020.

[17] V. Kloten, R. Lampignano, T. Krahn, and T. Schlange, "Circulating Tumor Cell PD-L1 Expression as Biomarker for Therapeutic Efficacy of Immune Checkpoint Inhibition in NSCLC," *Cells*, vol. 8, no. 8, pp. 1–12, 2019.

[18] M. Ilie *et al.*, "Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of issue for anti-PD-L1 therapeutic strategies," *Ann. Oncol.*, vol. 27, no. 1, pp. 147–153, 2015.

[19] N. Gettinger *et al.*, "Long-term Survival, Clinical Activity, and Safety of Nivolumab (Anti-PD-1; BMS-936558, ONO-4538) in Patients (Pts) With Advanced Non-Small Cell Lung Cancer (NSCLC)," *Int. J. Radiat. Oncol.*, vol.

328 90, no. 5, p. S34, 2014.
329 [20] R. Hatae *et al.*, “Combination of host immune metabolic biomarkers for the PD-1
330 blockade cancer immunotherapy,” *JCI Insight*, vol. 5, no. 2, pp. 1–18, 2020.
331 [21] D. R. Gandara *et al.*, “Blood-based tumor mutational burden as a predictor of
332 clinical benefit in non-small-cell lung cancer patients treated with atezolizumab,”
333 *Nat. Med.*, vol. 24, no. 9, 2018.
334 [22] D. T. Le *et al.*, “PD-1 Blockade in Tumors with Mismatch-Repair Deficiency,” *N.
335 Engl. J. Med.*, vol. 372, no. 26, pp. 2509–2520, 2015.
336 [23] M. Jiang *et al.*, “Peripheral Blood Biomarkers Associated With Outcome in
337 Non-small Cell Lung Cancer Patients Treated With Nivolumab and Durvalumab
338 Monotherapy,” *Front. Oncol.*, vol. 10, no. June, 2020.
339 [24] S. Diem *et al.*, “Neutrophil-to-Lymphocyte ratio (NLR) and
340 Platelet-to-Lymphocyte ratio (PLR) as prognostic markers in patients with
341 non-small cell lung cancer (NSCLC) treated with nivolumab,” *Lung Cancer*, vol.
342 111, no. December 2016, pp. 176–181, 2017.
343 [25] J. Liu *et al.*, “Systemic immune-inflammation index, neutrophil-to-lymphocyte
344 ratio, platelet-to-lymphocyte ratio can predict clinical outcomes in patients with
345 metastatic non-small-cell lung cancer treated with nivolumab,” *J. Clin. Lab.
346 Anal.*, vol. 33, no. 8, pp. 1–8, 2019.
347 [26] R. Schoppmeyer *et al.*, “Human profilin 1 is a negative regulator of CTL
348 mediated cell-killing and migration,” *Eur. J. Immunol.*, vol. 47, no. 9, pp.
349 1562–1572, 2017.
350 [27] M. Gagat, M. Hałas-Wiśniewska, W. Zielińska, M. Izdebska, D. Grzanka, and A.
351 Grzanka, “The effect of piperlongumine on endothelial and lung adenocarcinoma
352 cells with regulated expression of profilin-1,” *Onco. Targets. Ther.*, vol. Volume
353 11, pp. 8275–8292, Nov. 2018.
354 [28] H. Matsushita *et al.*, “Cancer exome analysis reveals a T-cell-dependent
355 mechanism of cancer immunoediting,” *Nature*, vol. 482, no. 7385, pp. 400–404,

2012.

[29] E. Zorn and T. Hercend, “A natural cytotoxic T cell response in a spontaneously regressing human melanoma targets a neoantigen resulting from a somatic point mutation,” *Eur. J. Immunol.*, vol. 29, no. 2, pp. 592–601, 1999.

[30] E. Giampazolias *et al.*, “Secreted gelsolin inhibits DNGR-1-dependent cross-presentation and cancer immunity,” *Cell*, pp. 1–16, 2021.

[31] P. Zaenker and M. R. Ziman, “Serologic autoantibodies as diagnostic cancer biomarkers - A review,” *Cancer Epidemiol. Biomarkers Prev.*, vol. 22, no. 12, pp. 2161–2181, 2013.

[32] L. B. Alexandrov *et al.*, “Signatures of mutational processes in human cancer,” *Nature*, vol. 500, no. 7463, pp. 415–421, 2013.

Figure caption

Fig. 1 (A) ROC curve of a set of four IC-antigens in serum before treatment (first set). (B) ROC curve of a set of four IC-antigens in serum after treatment (second set).

Table 1 Clinical characteristics of responders and non-responders.

	Responder	Non-responder
Number of Subjects	15	20
Age, mean ± SD, yrs	65.9 ± 7.30	69.5 ± 7.37
Sex, %female	25	25
Histology, %		
Adenocarcinoma	60	60
Squeamous cell	27	25
Other	13	15
EGFR mutations, %		
Positive	0	10
Unknown	0	10
ECOG PS, %		
1	93	100
2	7	0
TNM staging, %		
III	13	15
IV	67	80

ECOG PS = Esterm Cooperative Oncology Group performance status; EGFR = epidermal growth factor receptor; SD = standard deviation.

Table 2 Summary of serum IC-antigens characteristic of responders.

Accession	IC-antigen *	Responder (Frequency, %)	Non-responder (Frequency, %)
		Before treatment	
P07737	Profilin-1	47	10
P06733	Alpha-enolase	67	35
P00491	Purine nucleoside phosphorylase	33	5
P15531	Nucleoside diphosphate kinase A **	67	35
		After treatment	
P62937	Peptidyl-prolyl cis-trans isomerase A	73	40
P22314	Ubiquitin-like modifier-activating enzyme 1	27	0
P07358	Complement component C8 beta chain	47	20
O14791	Apolipoprotein L1	80	55

* Immune complex antigen, IC-antigen

** Detection frequency is calculated by combining frequencies of nucleoside diphosphate kinase A and putative nucleoside diphosphate kinase because sequence homology between them is very high (Blast search: coverage, 90%; identity; 85%).

Table 3 Sensitivity, specificity, PPV, and NPV of different cutoff values used in ROC analyses by the number of IC-antigens.

Number of IC-antigens	True positive	False positive	False negative	True negative	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Before treatment								
≧1	13	11	2	9	87	45	54.2	81.8
≧2	9	4	6	16	60	80	69.2	72.7
≧3	6	1	9	19	40	95	85.7	67.9
≧4	4	1	11	19	27	95	80.0	63.3
After treatment								
≧1	14	14	1	6	93	30	50.0	85.7
≧2	12	5	3	15	80	75	70.6	83.3
≧3	7	4	8	16	47	80	63.6	66.7
≧4	1	0	14	20	7	100	100.0	58.8

PPV = positive predictive value; NPV = negative predictive value

a) Number of IC-antigens included in the first set or the second set.