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# Interstitial pneumonia associated with MPO-ANCA: Clinicopathological features of nine patients

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**Summary**
Myeloperoxidase anti-neutrophil cytoplasmic autoantibody (MPO-ANCA) is a well known marker for small vessel vasculitis. Recent reports have demonstrated that interstitial pneumonia (IP) may rarely be associated with serum MPO-ANCA. Yet, little is known about the histological features.

We reviewed surgical lung biopsy from nine patients with IP of uncertain etiology with serum MPO-ANCA.

There was a male predominance (6:3) with a median age of 62.1. Histologically, eight patients presented with a usual interstitial pneumonia (UIP) pattern of pulmonary fibrosis, frequently accompanied by areas of nonspecific interstitial pneumonia (NSIP) pattern. One patient showed diffuse alveolar damage (DAD), and two patients showed mixture of UIP and DAD reflecting acute exacerbation of UIP. Microscopic honeycomb cysts were common, but fibroblastic foci were inconspicuous. The most frequent additional findings were small airway disease (9/9), and lymphoid follicles (7/9). Neither capillaritis nor vasculitis was seen in any of our cases. Three patients had microscopic hematuria, but none progressed to microscopic polyangiitis during the follow up. Mortality rate was 44% (median follow up 39.1 months).

IP associated with MPO-ANCA showed characteristic histology dominated by UIP pattern. Vasculitis was not identified in our cohort, but small airways disease and lymphoid follicles

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were present in most cases. IP associated with MPO-ANCA may be a histologically distinctive disease from idiopathic pulmonary fibrosis. Mortality was relatively high and life threatening acute exacerbation may occur.


## Introduction

Serum myeloperoxidase anti-neutrophil cytoplasmic autoantibody (MPO-ANCA) is not specific for any single disease and can be found in a variety of vasculitides, most commonly microscopic polyangiitis (MPA). However the precise biological mechanisms remain unknown.[1]

Recent reports have shown that some patients with interstitial pneumonia (IP) may have positive serum MPO-ANCA without developing known vasculitis. The number of publications on this topic is limited and the histological features of IP associated with serum MPO-ANCA have not been well-defined.[2] Based on existing reports, the majority of affected patients do not progress to MPA over the course of their disease.[3]

Newly published guidelines on the diagnosis and management of idiopathic pulmonary fibrosis (IPF) emphasize the importance of separating IPF from IP associated with specific systemic conditions such as those related to the collagen vascular diseases. If IP associated with MPO-ANCA is a distinctive form of pulmonary fibrosis, it should be separated from IPF. In that case, alternative therapy may be considered, especially since these individuals are not likely to qualify for clinical trials targeting IPF.[4]

In the present study, we describe the histopathologic findings and survival in a group of patients with IP associated with elevated serum MPO-ANCA.

## Materials and methods

The pulmonary pathology consultation files of the Department of Surgical pathology, Toyama University Hospital, from 2008 to 2011, were reviewed in order to identify cases that showed interstitial pneumonia accompanied by elevated levels of serum MPO-ANCA. Clinical data, including age, gender, smoking history, symptoms, existence of MPA and follow up data were obtained from patient medical records. Laboratory and pulmonary function data at the initial presentation were also recorded. All patients were evaluated for the presence of autoimmune antibodies including anti-nuclear antibody (ANA), MPO-ANCA, and proteinase-3 (PR3)-ANCA. According to the Japanese guideline for idiopathic interstitial pneumonias,[5] basically all interstitial pneumonia cases are recommended to examine serum ANA, MPO-ANCA, PR3-ANCA and other autoimmune antibody as a routine screening test to exclude the collagen vascular disease from idiopathic interstitial pneumonias. In the guideline, the serum MPO-ANCA level of $>$20 EU is suggested to be positive.

*Radiological review*: high-resolution computed tomography (HRCT) scans of the chest at the time of biopsy were available for all patients. The HRCT scans were reviewed in a blinded fashion by a chest radiologist (R.E.) experienced in the interpretation of diffuse lung disease and were classified using the radiologic patterns described in the ATS/ERS International Consensus Classification 2002 of the Idiopathic Interstitial Pneumonias (IIPs).[6]

*Histologic review*: all lung biopsy specimens were reviewed independently by two pathologists (J.F., T.T.). Each biopsy was classified using the histopathologic patterns described in the ATS/ERS International Consensus Classification 2002 of the IIPs. In cases where there was disagreement, a consensus was established through further review. The following histologic features were semi-quantitatively graded: marked dense fibrosis (MF: $-$, absent; $+$ weak; $++$, strong), patchy fibrosis (PF: $-$, absent; $+$ weak; $++$, strong), honeycomb change (HC: $-$, absent; $+$, weak; $++$, strong), fibroblastic foci (FF: $-$, absent; $+$, weak; $++$, strong), capillaritis (CA: $-$, absent; $+$ weak; $++$, strong), vasculitis (VA: $-$, absent; $+$ weak; $++$, strong), lymphoid follicle with germinal center (LY: $-$, absent; $+$, weak; $++$,strong), small airway disease (SAD: $-$, absent; $+$, weak; $++$, strong). When small airway diseases were observed, they were divided into three subtypes, cellular, cellular and fibrotic, and fibrotic, depending on the predominance of inflammation and fibrosis. Follicular bronchiolitis was included in cellular bronchiolitis.

If other relevant histological findings (e.g. granuloma) were present, they were also documented. The evaluation for capillaritis and vasculitis was performed using slides stained with H&E and elastica van Gieson (EVG) stainings.

To understand the clinical outcome, a Kaplan Meier curve was plotted, and a median survival time was pointed out.

The study protocol was approved by the Institutional Review Board of Toyama University Hospital.

## Results

### Clinical findings

Among total of 272 cases sent from the three institutes for consultation to the pathology archive of interstitial pneumonia at Toyama University Hospital, 224 patients were available for MPO-ANCA levels at the time of biopsy. Out of those 224 patients, we identified nine patients showing higher levels of MPO-ANCA. PR3-ANCA levels were also tested for majority of patients at the time of biopsy, which showed 4 out of 219 patients were positive. Only one patient, patient 2, showed positive for both MPO and PR3-ANCA. Data of other autoimmune antibodies were also available at the time of biopsy, which showed positive for RF, 50/222; ANA, 41/227; SS-A, 20/216; Jo-1, 10/231; RNP, 6/151; Scl-70, 2/215 and SS-B, 1/194.The mean age of elevated serum MPO-ANCA patients was 62.1 years with a male predominance (M:F = 6:3). Three patients demonstrated microscopic hematuria, while two patients who were detected on a health check-up had no symptoms. A

pulmonary function test revealed that six of the nine patients had restrictive impairment. Follow up ranged from 19 to 72 months (mean 39.1). Four patients died during the follow up period; two of whom had acute exacerbation. (Table 1) Median survival time was 46 months (Fig. 3). With available follow up, none of the patients in our study progressed to MPA. Also, the three patients who showed microscopic hematuria did not progress to MPA during the period of follow up. No patients had renal biopsy because the renal symptoms, if any, were subtle. The laboratory findings including the levels of serum MPO-ANCA were shown in Table 2.

## Radiological findings

HRCT scans were available for all patients and radiographic patterns of each case are shown in Table 2. The most common findings were bilateral reticulo-nodular shadows superimposed on ground glass opacities. CT findings were classified as presenting a UIP pattern in six cases, showing honeycomb change with reticular abnormality (Fig. 2A). Patient 3 showed an NSIP pattern with predominant ground glass opacity without honeycomb change. Patient 1 showed organizing pneumonia (OP) with patchy distribution of consolidation. Patient 8 showed a mixture of reticulo-nodular shadows and diffuse ground glass findings consistent with acute exacerbation of background interstitial fibrosis (Fig. 2B). Patient 9 showed ground glass opacity with traction bronchioloectasis, suspicious of DAD.

## Histological findings

Biopsies for each patient were obtained from one to three sites of the lung. Histological features are summarized in Table 3. The histological investigation revealed a UIP pattern in eight out of nine patients. All UIP cases showed marked dense fibrosis in mostly peripheral areas of secondary lobules. Seven out of eight UIP cases showed honeycomb changes in which the sizes of the cysts were up to 10 mm in diameter (Fig. 1A–C). Fibroblastic foci were inconspicuous in six out of eight UIP cases, while two cases showed scattered foci. Most UIP patients had NSIP-like areas as a minor component of their disease, with uniform distribution of fibrosis. Two patients exhibited UIP with superimposed DAD (Fig. 1D). The two patients were suspected histologically of having acute exacerbation of chronic fibrosing interstitial pneumonia. A CT scan of the same case confirmed the DAD radiologically, and a clinical–radiological–pathological (CRP) consensus diagnosis of acute exacerbation of UIP was established. Another patient, case 1, showed DAD in the organizing phase.

An additional frequent and characteristic finding commonly seen in IP associated with MPO-ANCA is small airway disease. Three of the nine patients showed cellular bronchiolitis exhibiting lymphoplasmacytic infiltration around the airway with little fibrosis (Fig. 1E) in which two accompanied lymphoid follicles with germinal center, showing features of follicular bronchiolitis. Five of the nine patients showed combined cellular and fibrotic bronchiolitis. One patient demonstrated purely fibrotic bronchiolitis. One patient (Patient 3) also showed mild constrictive bronchiolar fibrosis on top of cellular bronchiolitis in some membranous bronchioles.

Another characteristic finding in the current study was the presence of lymphoid follicles with a germinal center (Fig. 1A, B, E). Seven of the nine patients showed lymphoid follicles with a germinal center. No patient showed evidence of capillaritis or vasculitis, however, two patients showed mild lymphocytic infiltration inside the vessel walls (lymphocytic vasculopathy) (Fig. 1F). Case 2 showed a single non-necrotizing, poorly formed granuloma, which was considered as an incidental finding.

## Discussion

The present study has shown that interstitial pneumonia of unknown cause with elevated serum MPO-ANCA exhibits characteristic histopathology dominated by advanced fibrous remodeling of UIP pattern. Capillaritis and/or vasculitis were not identified but small airway disease and lymphoid follicles were commonly seen in our study. The prognosis seems to be poor. Frequency of acute exacerbation is not clear but may be as high as IPF.[4]

**Table 1** Patients demographics of Interstitial Pneumonia associated with MPO-ANCA.

| | Age | Gender | Symptom | MPA | AE | Treatment | Follow up (Mo) | Status |
|---|---|---|---|---|---|---|---|---|
| Patient 1 | 59 | F | Fever, arthralgia | No | − | PSL | 48 | Dead |
| Patient 2 | 71 | M | DOE, bloody sputum, recurrent fever | No | − | PSL + CyA | 25 | Alive |
| Patient 3 | 69 | M | DOE, microscopic hematuria | No | − | PSL + CyA | 72 | Alive |
| Patient 4 | 58 | F | DOE, microscopic hematuria | No | + | PSL + CyA | 35 | Dead |
| Patient 5 | 62 | F | None[a] | No | − | Untreated | 21 | Alive |
| Patient 6 | 59 | M | Dry cough | No | + | PSL + CyA | 33 | Dead |
| Patient 7 | 54 | M | Pancreatitis | No | − | PSL + CyA | 19 | Alive |
| Patient 8 | 58 | M | Microscopic hematuria | No | + | PSL + CyA | 53 | Alive |
| Patient 9 | 69 | M | None[a] | NA | − | PSL + CyA | 46 | Dead |

MPA, microscopic polyangiitis; AE, acute exacerbation of chronic disease; DOE, dyspnea on exertion; PSL, prednisolone; CyA, cyclosporine A; NA, data not available.

[a] These patients' diseases were found by health check.

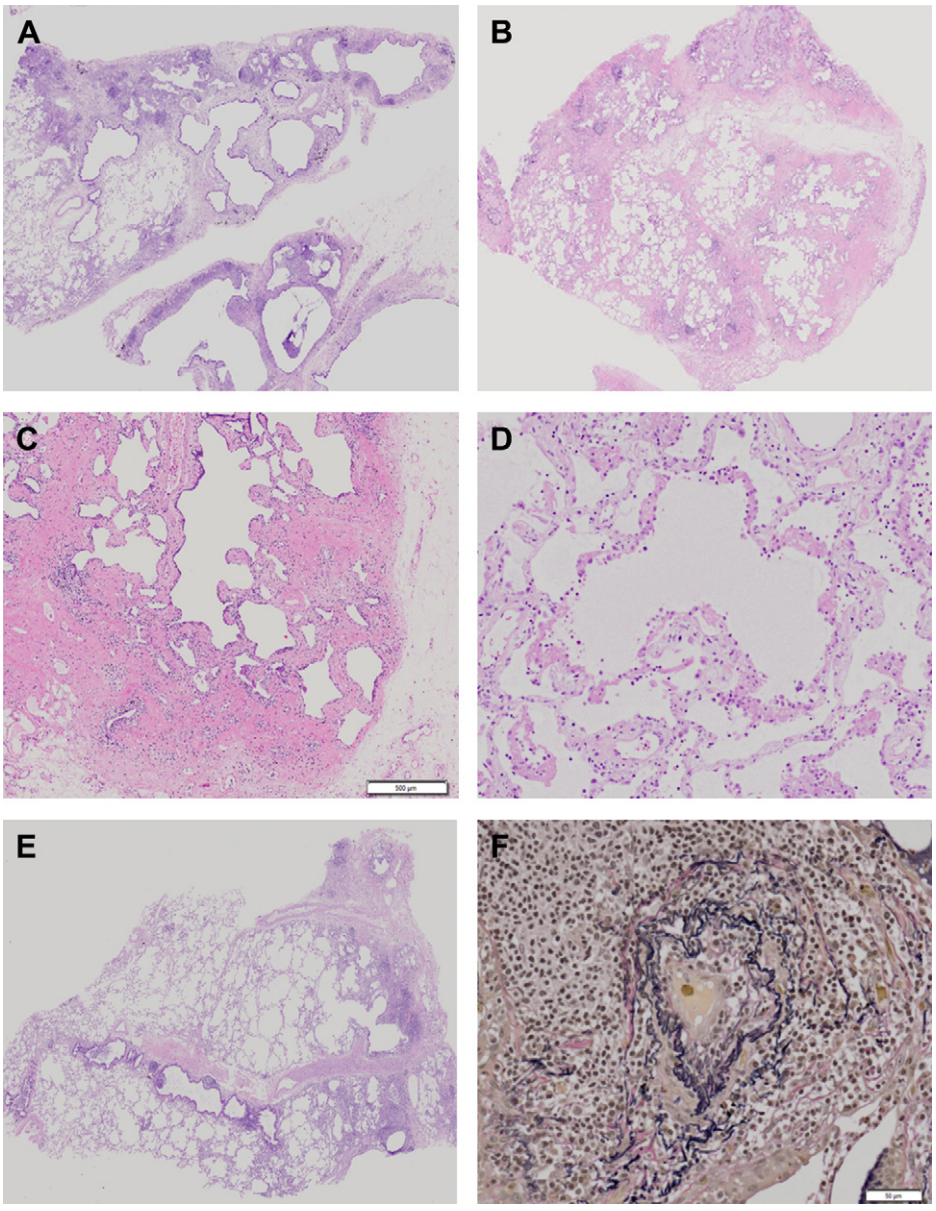


**Figure 1** Histopathological features of Interstitial Pneumonia Associated with MPO-ANCA. (A) Lower power view (×1) of patient 2. Large cystic spaces possibly honeycombing can be seen. Basic lung architecture is totally distorted (H&E stain). (B) Lower power view (×1) of Patient 8 shows patchy dense fibrosis accentuated in the peripheral zones of the lobules corresponding to UIP pattern (H&E stain). (C) Higher power view (×4) of the same case shows dense collagenous fibrosis with architectural destruction along with cystic spaces possibly microscopic honeycombing (H&E stain). (D) Another lobe of the patients 8 showed hyaline membranes indicating DAD (H&E staining, ×10). (E) Low power view (×1) of another lobe of the patients 2 showed small airway disease (H&E stain). (F) High power view of same lobe revealed vasculopathy. Note preservation of elastic lamina in the vascular wall (EVG stain, ×20).

Collagen vascular diseases (CVD) are known to produce pleuropulmonary manifestations. For most of the CVD, except rheumatoid arthritis, NSIP histology is a common manifestation.[7,8] In the present study, eight out of nine patients had UIP pattern as a major histology. Our data, the predominance of UIP in IP associated with MPO-ANCA, was identical to previous reports.[3] UIP histology may be a characteristic presentation of IP associated with MPO-ANCA. However, the vast majority of our cases showed a significant mixture of NSIP patterns in the same biopsy. Similar to IP associated with CVD, the atypical UIP histology may be a histological features of IP associated with MPO-ANCA.

Recently interstitial pneumonia with highly suspected relation to collagen vascular disease but not fulfilling the criteria of defined collagen vascular disease is categorized under three different names: undifferentiated connective tissue disease (UCTD),[9,10] lung dominant connective tissue disease,[11] and autoimmune-featured interstitial lung disease.[12] Among them, serum ANCA was included in the criteria of autoimmune-featured interstitial lung disease

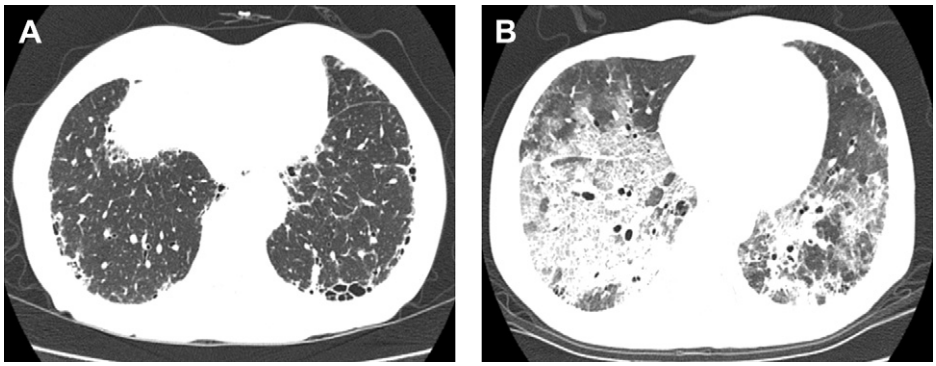


**Figure 2** CT findings of Interstitial Pneumonia Associated with MPO-ANCA Patient 2 (A) showed relatively large sized cyst and honeycomb change at subpleural predominance in the lung base. Patient 8 (B) demonstrated reticulo-nodular shadows as a background finding superimposed of diffuse ground glass opacity corresponding to acute exacerbation.

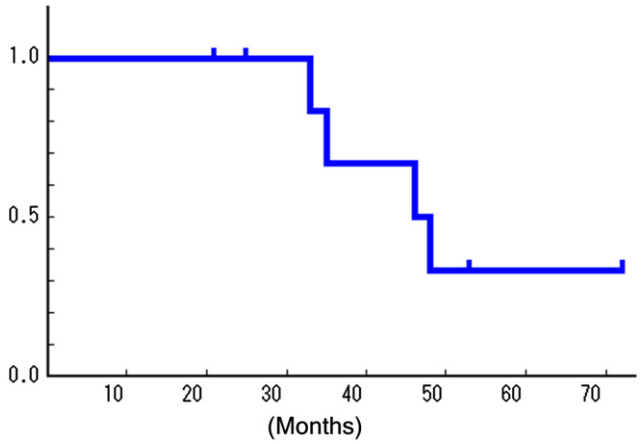


**Figure 3** Kaplan Meier curve of patients with interstitial pneumonia associated with MPO-ANCA. Eight out of nine patients had follow up data. A Kaplan Meier curve was plotted. All four cases experiencing death of the disease were due to acute exacerbation of chronic interstitial pneumonia of usual interstitial pneumonia. Median survival time of all patients was 46 months.

without clear evidence as background. Fischer et al. proposed four histological criteria for lung dominant connective tissue disease; most cases of the present study suit at least one of the criteria, lymphoid follicle with germinal center.[11] Also, all patients in the present study showed small airway disease in which cellular and follicular bronchiolitis were frequent histological patterns. Small airway disease is also common in IP associated with CVD.[11,13] Considering these overlaps with CVD, interstitial pneumonia associated with serum MPO-ANCA may fit better within the context of connective tissue disease with related interstitial lung disease than with IPF that has an incidental serum elevation of MPO-ANCA.[14]

Our study indicates that the histopathology of IP associated with MPO-ANCA is discernibly different from that of IPF and suggests that these cases may be regarded and treated more like a systemic rheumatic disease manifesting in the lung.

In the report of Homma et al., 13 of the 31 patients died of deteriorations related to the disease.[3] The occurrence of DAD in a subset of our study patients is important and suggests that patients with IP associated with MPO-ANCA may be at relatively high risk for acute exacerbation.

This time, we focused on interstitial pneumonia associated with MPO-ANCA. Overlap and differences of clinicopathological features from other auto-antibodies are not known. Further studies to investigate exact relationship between types of autoantibody and clinicopathological data are needed.

A limitation of the current study is the small number of patients and the retrospective nature of our case identification. Further studies will be required with a larger number of patients to confirm our observations.

**Table 2** Smoking status, laboratory findings and CT features.

| | Smoking | Pack year | MPO-ANCA [EU] | Autoantibody | KL-6 [U/ml] | %VC | %FEV1.0 | %Dlco | CT pattern |
|---|---|---|---|---|---|---|---|---|---|
| Patient 1 | Never | 0 | 24 | Negative | 187 | 68.1 | NA | 76.1 | OP |
| Patient 2 | Ex | 45 | 35.2 | Negative | 588 | 69.9 | 83.0 | 69.7 | UIP |
| Patient 3 | Ex | 50 | 90 | ANA(×640) | 2120 | 74.3 | NA | 50.0 | NSIP |
| Patient 4 | Never | 0 | Positive[a] | Negative | 2200 | 56.0 | 74.2 | 32.3 | UIP |
| Patient 5 | Never | 0 | 205.0 | Negative | 339 | 104.7 | 105.9 | 80.4 | UIP |
| Patient 6 | Ex | 15 | 31 | ANA(×320) | 1743 | 102.8 | 94.0 | 91.8 | UIP |
| Patient 7 | Ex | 42 | 34.9 | ANA(×640) | 508 | 74.2 | 74.0 | NA | UIP |
| Patient 8 | Current | 57 | 39.1 | Negative | 714 | 52.9 | 51.5 | 26.2 | UIP |
| Patient 9 | Ex | 90 | Positive | ANA(×160) | NA | 98.1 | 106.6 | 81.5 | DAD |

Ex, ex-smoker; NA, data not available.
[a] Exact level of MPO-ANCA was not given.

**Table 3** Histological findings of interstitial pneumonia associated with MPO-ANCA.

| | Pathologic pattern | MF | PF | HC | FF | Capillaritis/ vasculitis | Vasculopathy | Lymphoid follicle | Small airway disease | (Subtype) | Other findings |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient 1 | DAD | − | − | − | − | − | − | − | + | F | |
| Patient 2 | UIP | + | ++ | ++ | − | − | + | ++ | ++ | C | Granuloma |
| Patient 3 | UIP | ++ | + | + | − | − | + | ++ | + | C (FB) + F | Constrictive change |
| Patient 4 | UIP | + | ++ | +/− | ++ | − | − | + | + | C + F | |
| Patient 5 | UIP | + | + | + | − | − | − | ++ | ++ | C (FB) + F | |
| Patient 6 | UIP | ++ | + | ++ | − | − | − | ++ | ++ | C | |
| Patient 7 | UIP | ++ | + | ++ | − | − | − | + | + | C | |
| Patient 8 | UIP + DAD | ++ | + | − | − | − | − | + | + | C + F | |
| Patient 9 | UIP + DAD | + | + | − | + | − | − | − | + | C + F | |

MF, marked dense fibrosis; PF, patchy fibrosis; HC, microscopic honeycomb cyst; FF, fibroblastic focus; SAD, small airway disease; C, cellular bronchiolitis; C + F, cellular and fibrotic bronchiolitis; F, fibrotic bronchiolitis; C (FB), follicular bronchiolitis.

## Conclusions

Interstitial pneumonia associated with MPO-ANCA has characteristic histopathology dominated by usual interstitial pneumonia pattern. Bronchiolitis and lymphoid follicles were common as in connective tissue disease associated with interstitial lung disease. Interstitial pneumonia associated with MPO-ANCA may be a distinctive disease from idiopathic pulmonary fibrosis. The prognosis seems to be poor, and lethal acute exacerbation may occur.

## Disclosure/conflict of interests

(1) Ownership: Junya Fukuoka is a representative of a venture company founded inside Toyama University, Pathology Institute Corporation, and holds stocks of the company.
(2) Income: no one does receive $10,000 or more of income per annum from any single private or public company in the health care field.
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(4) No other authors have potential conflict of interests related to the present work.

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