Long-term outcomes of patients with propylthiouracil-induced anti-neutrophil cytoplasmic auto-antibody-associated vasculitis

Y. Gao¹,², M. Chen¹, H. Ye², F. Yu¹, X.-h. Guo² and M.-h. Zhao¹

Objective. It was well known that propylthiouracil (PTU) could induce ANCA-associated vasculitis (AAV) and clinical evident vasculitis could resolve after cessation of PTU with or without immunosuppressive therapy. However, the treatment strategy for patients with PTU-induced AAV remained inconclusive and their long-term outcomes were lacking. The aim of our study was to summarize these data.

Methods. Fifteen patients with PTU-induced AAV, receiving immunosuppressive agents for <12 months and following over 24 months, were selected in the current study. The clinical and pathological data, including treatment protocols and outcomes, were retrospectively investigated.

Results. All the patients were followed for a mean of 55.0 (25–98) months. PTU was discontinued upon diagnosis of PTU-induced AAV. Immunosuppressive therapy was administrated only for patients with vital organ involvements, such as lung and kidney, and lasted only 7.9 ± 3.3 (0.27–12) months. No relapse of vasculitis occurred during follow-up, even after withdrawal of immunosuppressive therapy. Twelve (80%) patients remained in complete remission and one patient remained in partial remission at the latest follow-up. Two patients were treatment resistant due to late referral and late withdrawal of PTU, both of them progressed to end-stage renal disease. For uncontrolled hyperthyroidism on presentation, six patients switched to methimazole and none of them experienced relapse of vasculitis.

Conclusions. The long-term outcomes of patients with PTU-induced AAV were relatively good. PTU should be discontinued immediately after diagnosis. Immunosuppressive therapy may be only used in patients with vital organ involvements, and a long-term maintenance therapy may not be necessary.

Key words: Propylthiouracil, Vasculitis, Antineutrophil cytoplasmic antibodies, Hyperthyroidism, Therapy, Prognosis.

Introduction

ANCA-associated vasculitis (AAV) is associated with high morbidity and mortality, as well as potentially life-threatening toxicity from immunosuppressive therapy. Thus, identification of potentially reversible causes of AAV, such as specific drugs, is very important [1]. Propylthiouracil (PTU) is a common anti-thyroid drug, which has been known to induce AAV [2–6] with multiple ANCA antigen specificities [7].

Although immunosuppressive therapy remained mainstay in patients with primary AAV, it has been reported that clinical evident vasculitis of PTU-induced AAV could resolve merely after cessation of PTU without immunosuppressive therapy, even in patients with severe organ involvement such as alveolar haemorrhage [8]. However, it was reported that PTU-induced AAV might be fatal in spite of intensive immunosuppressive therapy in some cases [9]. Therefore, treatment strategy for patients with PTU-induced AAV remains inconclusive. Furthermore, it has been well known that patients with primary AAV should receive maintenance therapy for years after achieving remission to control frequent relapses of vasculitis. Whether the maintenance therapy should be administrated in the same way in patients with PTU-induced AAV has not been determined yet. Most patients with PTU-induced AAV might take unnecessary risks in the long-term immunosuppressive therapy.

Regarding to the different pathogenesis between PTU-induced AAV and primary vasculitis, we recommended different therapeutic protocols for patients with PTU-induced AAV. PTU should be discontinued immediately after diagnosis of PTU-induced AAV; immunosuppressive therapy should be administrated only for patients with vital organ involvement, such as lung and kidney vasculitis. Furthermore, the immunosuppressive therapy only lasted for 6–12 months, according to disease severity, without further maintenance therapy. In the current study, we summarized the treatment protocols and long-term outcomes of 15 patients with PTU-induced AAV treated with the above protocols.

Materials and methods

Patients

Fifteen patients with PTU-induced AAV, diagnosed in Peking University First Hospital during December 1999 to December 2005, were selected in the current study. They were followed up for at least 24 months. All these patients fulfilled the 1994 Chapel Hill Consensus Conference definition for AAV. PTU-induced AAV was defined as following: (i) the signs and symptoms of vasculitis were temporally related to using PTU, and regressed with its discontinuation; (ii) serum ANCA was positive, especially those with multi-antigenicity; (iii) medical conditions that mimicked vasculitis were excluded, especially infections and malignancies, and other definable types of vasculitis [10].

Patients with hyperthyroidism were diagnosed on the basis of typical clinical manifestations, elevated serum-free triiodothyronine (FT3) and free thyroxine (FT4) and very low or undetectable thyrotropin. The diagnosis of Graves’ disease was based on the fulfilment of at least one of the following classical criteria: ophthalmopathy; a diffuse goitre; pre-tibial myxoedema and the presence of thyrotropin receptor antibody.

This study was complied with the Helsinki declaration and was approved by the Ethics Committee of Peking University First Hospital. All patients gave written informed consent. Data on clinical manifestation and laboratory results were collected upon diagnosis and during follow-up. All the patients were revisited every 1–3 months in the first 6 months after diagnosis of PTU-induced AAV, and every 3–6 months during subsequent follow-up.

ANCA analysis

Sequential serum samples were collected upon diagnosis before immunosuppressive treatment, and about every 3 months in the
first 6 months, every 6–12 months during subsequent follow-up. ANCA were detected by both indirect IF (IIF) assay and antigen-specific ELISAs as described [11, 12].

IIF assay. All sera were screened for ANCA by IIF technique according to the manufacturer (Euroimmun, Lübeck, Germany). The fluorescence patterns were classified as cytoplasmic ANCA (C-ANCA) and perinuclear ANCA (P-ANCA). Slides were read by two independent observers who were not aware of the clinical diagnosis.

Antigen-specific ELISA. Seven highly purified known ANCA antigens, including MPO, proteinase 3, human leucocyte elastase (HLE), lactoferrin, cathepsin G, azurocidin and bactericidal/permeability-increasing protein (BPI) [13–16], were initially used as solid-phase ligands to detect ANCA specificities as previously described [7], and antigen-specific ELISAs to MPO and proteinase 3 were also used to detect sequential sera during follow-up.

Thyroid function
Serum FT3, FT4 and thyrotropin were measured with chemoluminescence assay (Chiron Diagnostics ACS 180 Plus, MA, USA).

Organ involvement of PTU-induced AAV
Organ involvements such as lung, renal, neurologic and ENT involvement and gastrointestinal vasculitis were determined by biopsy or by well-defined clinical criteria [17, 18]. Systemic involvement included fever, general malaise and weight loss. Cutaneous disease was defined by a characteristic palpable purpuric rash or urticaria [19] with or without ulcerations and/or pathologically confirmed leucocytoclastic angiitis.

Definitions of organ involvement
Haematuria was graded as ‘gross’ or ‘positive’ (five or more red blood cells per high-power field). Proteinuria was measured by the pyrogallol red method and evaluated by 24 h quantitative measurement. The modified modification of diet in renal disease equation in Chinese patients [20] or Schwartz equation [21] was used to estimate glomerular filtration rate (eGFR) in patients older or less than 18 yrs, respectively. End-stage renal disease (ESRD) was defined when a patient required chronic maintenance dialysis or renal transplantation. A euthyroid state was defined when serum levels of FT3, FT4 and thyrotropin were within normal ranges.

Treatment categories
Treatment protocols varied in patients and were drawn up in Fig. 1. Treatment for patients with systemic symptoms only and without lung or kidney involvement, was merely discontinuation of PTU. Therapy for the patients with renal involvement depended on the severity of clinical manifestation and histopathological lesions. Prednisone was given at an initial dose of 1 mg/kg/day for the first 4–8 weeks, followed by a gradually tapering dose within 6–12 months. Cyclophosphamide (0.6–1.0 g/month intravenously, or 1–2 mg/kg/day orally) or mycophenolate mofetil (1.5–2.0 g/day) were administrated for 6–12 months. In addition, patients with severe necrotizing crescentic GN and diffuse pulmonary alveolar haemorrhage were also treated with pulse methylprednisolone (7–15 mg/kg/day) for 3 days, and patients with life threatening massive pulmonary haemorrhage were treated with plasmapheresis as well [22].

Treatment response
Treatment response [17, 18, 23] in patients with PTU-induced AAV was defined as follows: remission was defined as stabilization or improvement of renal function by calculated eGFR and resolution of other manifestations of systemic vasculitis for >1 month [24], and it was further defined as complete remission and partial remission. Complete remission was indicated by normalization of renal function if renal insufficiency is existing and by resolution of haematuria and extra-renal manifestations of systemic vasculitis. Partial remission was defined by stabilization or improvement of renal function or dialysis being independent if renal insufficiency existed or by resolution of haematuria and proteinuria and/or resolution of extra-renal manifestations of systemic vasculitis. Treatment resistance was defined as progressive decline in kidney function with persistence of active urine sediment, or new or persisting extrarenal manifestations of vasculitis despite immunosuppressive therapy. Relapse could only occur in patients who reached remission and it was defined as the reoccurrence of vasculitic signs or symptoms in any organ system.

Statistical analysis
Data were presented as mean ± s.d., unless otherwise indicated.

Results
Demographic characteristics of patients with PTU-induced AAV on diagnosis
As shown in Table 1, 14 patients with PTU-induced AAV were female and the other one was male with a mean age of 29.7 ± 13.9 (9–57) yrs. All patients were diagnosed with Graves’ disease originally. They were not overlapped with other connective tissue diseases on diagnosis, and one patient had vitiligo for 20 yrs.

Before diagnosis of vasculitis, PTU had been used continuously except that one patient had restarted PTU due to relapse of hyperthyroidism. The duration of PTU therapy before its withdrawal was 48.0 ± 25.8 (1.6–96) months and 14/15 (93.3%) patients had received PTU for >24 months. The interval between the occurrence of clinical vasculitis and the diagnosis of PTU-induced AAV was 7.5 ± 4.9 (1–18) months. Patients were followed for 55.0 ± 22.1 (25–98) months.

Clinical manifestations on diagnosis
The clinical data on diagnosis were shown in Table 2. Two patients merely had systemic symptoms, 13/15 (86.7%) patients had renal involvement and 6/15 (40.0%) presented lung involvement.

P-ANCA was detected in sera from 14 (93.3%) patients and C-ANCA in one. All sera recognized MPO and four sera also recognized PR3. Antibodies directed against HLE, lactoferrin, cathepsin G, azurocidin and BPI occurred in 7/14 (50%), 10/14 (71.4%), 9/14 (64.3%), 8/14 (57.1%) and 0/14 (0%), respectively, and 13/14 (92.9%) recognized more than one target antigen.

As shown in Table 2, 14 (93.3%) patients had elevated ESR on diagnosis. ANA was detected in sera from two patients (13.3%); none of them had anti-dsDNA antibodies. Haematuria was observed in all the 13 patients with renal involvement, seven patients (53.8%) had gross haematuria. A 24 h urine collection showed pronounced proteinuria exceeding 3.5 g/day, only in two out of nine patients with proteinuria; both of them had acute renal failure upon diagnosis. Decreased eGFR (50 ml/min) was also detected in another patient.

Histopathological manifestations on diagnosis
Renal biopsy was performed in 9 of the 13 (69.2%) patients with renal involvement. Two were with focal segmental necrotizing GN, three were with necrotizing crescentic GN and the other four were with minor glomerular lesion. Immune complex was found in five out of the nine patients (55.6%) with renal biopsy.
One patient who refused renal biopsy received skin biopsy, which indicated early stage of pyoderma gangrenosum.

**Treatment of vasculitis**

As shown in Fig. 1, PTU was discontinued on diagnosis in all the patients. The two patients without organ involvement did not receive any further immnosuppressive therapy. Six patients with haematuria and without renal insufficiency (three had minor

<table>
<thead>
<tr>
<th>No.</th>
<th>Gender</th>
<th>Age (yrs)</th>
<th>Duration of PTU therapy (months)</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>34</td>
<td>96</td>
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<tr>
<td>4</td>
<td>F</td>
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<td>1.6</td>
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<tr>
<td>5</td>
<td>F</td>
<td>30</td>
<td>37</td>
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<td>6</td>
<td>F</td>
<td>9</td>
<td>32</td>
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<td>7</td>
<td>F</td>
<td>18</td>
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<td>20</td>
<td>60</td>
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<tr>
<td>15</td>
<td>F</td>
<td>32</td>
<td>36</td>
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</table>

F, female; M, male.

One patient who refused renal biopsy received skin biopsy, which indicated early stage of pyoderma gangrenosum.
lesion confirmed by renal biopsy, one received skin biopsy and the other two refused biopsy) only received oral prednisone, but one of the six patients refused to take prednisone further after 8 days. Combined therapy with oral prednisone and immunosuppressant was performed in one patient with diffuse pulmonary infiltrates and renal involvement but without renal biopsy. Six patients with severe necrotizing crescentic GN or focal segmental necrotizing GN or diffuse pulmonary alveolar hemorrhage received pulse methylprednisolone followed by combined oral prednisone and immunosuppressant, one of them also had plasmapheresis due to massive pulmonary haemorrhage. The duration of prednisone administration and immunosuppressant therapy was 7.9 ± 3.3 (0.27–12) months and 4.5 ± 3.1 (2–9) months, respectively, except that two patients received immunosuppressive therapy constantly due to renal transplantation.

Treatment efficacy and long-term outcomes of vasculitis
Throughout the follow-up, 12 patients remained in complete remission, one patient achieved and remained in partial remission, and the decreased eGFR was stable and haematuria still existed at the latest follow-up. Two patients were treatment resistant due to late withdrawal of PTU because of unawareness of PTU-induced AAV; both of them progressed to ESRD, and received dialysis and further renal transplantation.

After withdrawal of PTU, the non-specific signs and symptoms improved rapidly during the first week. Haematuria in one patient with normal renal function relieved only after cessation of PTU and an 8-day prednisone therapy. The mean duration for resolution of haematuria was 12.1 ± 13.1 (2–48) months. The initial eGFR in the two patients with ESRD was < 5 ml/min, and > 80% of glomeruli had fibrous or fibrocellular crescent formation in their renal histopathology.

All the 15 patients were followed up for a mean of 55.0 (25–98) months. No relapse of vasculitis occurred even after discontinuation of immunosuppressive therapy during follow-up.

Changes in ANCA levels during follow-up
For majority of patients with PTU-induced AAV, their ANCA levels declined gradually but sera ANCA remained positive in 11 patients for a long time, despite quiescence of clinical symptoms. ANCA turning negative only occurred in four patients (26.7%), and the duration between the start of treatment and ANCA turning negative was 24.8 ± 18.2 (3–45) months.

Treatments and long-term outcomes of hyperthyroidism
For nine patients with uncontrolled hyperthyroidism on diagnosis of PTU-induced AAV, six were switched to methimazole (MMI), one patient received subtotal thyroidectomy and the other two were treated with radioactive iodine. The other six patients had no further anti-thyroid treatment initially due to euthyroid state.

During follow-up, one out of the six patients with MMI therapy and one patient without any further anti-thyroid therapy at presentation experienced relapse of hyperthyroidism and then changed to radioiodine therapy. As shown in Table 3, at the latest follow-up, seven patients were euthyroid and five had hypothyroidism. Three patients remained to have mild hyperthyroidism or subclinical hyperthyroidism.

Interestingly, none of the patients with further MMI treatment had relapse of vasculitis during follow-up.

Discussion
The clinical manifestations of PTU-induced AAV are similar to those of other idiopathic vasculitides, which ranged from systemic manifestations to life-threatening multisystem vasculitis. In our study, the duration of drug exposure in majority patients (93.3%) was longer than 24 months; we speculated that longer treatments might be a risk factor for PTU-induced AAV [22]. Relapse of hyperthyroidism was reported as another risk factor [19]; however, we did not observe the similar phenomenon.

There are no unique clinically pathological or laboratory aspects of drug-induced vasculitis that help to differentiate it from other vasculitides [10]. In our previous studies, it had been shown that antibodies against multiple ANCA-specific antigens, especially the antigens rather than MPO and PR3, might be the characteristic feature of drug-induced ANCA, which might be helpful to discriminate it from primary AAV [7]. In the current study, 13/14 sera recognized more than one target antigen.

Our follow-up data were encouraging. None of our patients experienced relapse of vasculitis for a mean of 55.0 (25–98) months even after discontinuation of immunosuppressive therapy. It was quite different from the long-term outcomes of patients with primary AAV, because ~11–57% of patients with primary AAV had a relapse after achieving remission [24].

Since the mechanism of PTU-induced AAV was different from that of primary vasculitis, the cornerstone of treatment for primary AAV, including induction therapy might not be suitable for all the patients with PTU-induced vasculitis [22]. It had been suggested that PTU was an important factor involved in the pathogenesis and it might be involved in the production of ANCA [25]; therefore, cessation of PTU was essential in treatment for all the patients, and it might be also enough for those with non-specific systemic symptoms. However, in our previous study, one patient failed to withdraw PTU in time on diagnosis, his renal function deteriorated rapidly and he progressed to ESRD eventually [26]. It suggested that continued administration of PTU might be associated with a poor prognosis in patients with PTU-induced AAV. Nearly half (6/15) of the patients in our study had non-specific systemic symptoms preceding organ involvement. As Morita et al. [27] had suggested more severe specific organ involvement might develop in patients with non-specific systemic syndrome when PTU treatment was not withdrawn in time. Therefore, it is reasonable to recommend that PTU should be discontinued immediately after diagnosis of PTU-induced AAV [4].

In some case reports, organ involvement could also resolve in patients only after discontinuation of PTU. However, in another case report vasculitis worsened in the next 5 months after cessation of PTU [28]. Furthermore, in the literature, it was reported that at least five patients died of PTU-induced AAV in spite of intensive immunosuppressive therapy [9, 29]. Our data supported the notion that the appropriate treatment for PTU-induced AAV should be drawn up according to disease severity. We suggested that in patients with severe and active organ involvements, intensive immunosuppressive therapy such as corticosteroid and immunosuppressive agents should be administrated to improve organ function and to prevent progression to severe, irreversible disease. However, in spite of intensive therapy, renal function did not return to normal range in two patients with late crescentic GN in our study. As Gunton et al. [22] had mentioned if necrotizing crescentic GN was present, the patients were at high risk of developing chronic renal failure.

<table>
<thead>
<tr>
<th>Further thyroid treatment</th>
<th>Hypothyroidism (n)</th>
<th>Euthyroidism (n)</th>
<th>Subclinical Hyperthyroidism (n)</th>
<th>Hyperthyroidism (n)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No therapy</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>5</td>
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<tr>
<td>MMI subset</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>5</td>
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<td>5</td>
<td>11</td>
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<tr>
<td>Thyroidectomy</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<td>3</td>
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<tr>
<td>Radioactive iodine</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>15</td>
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</table>
Up to now, no clear-cut decisions have been reached on how long is the optimal period of immunosuppressive therapy and whether long-term maintenance therapy for patients with PTU-induced vasculitis is needed. In Fujieda’s report, although none of the seven Japanese children had relapse of PTU-induced AAV after immunosuppressive therapy, the mean duration of prednisolone administration was 32 ± 24 (range 1.0–60) months [30]. Helfgott and Smith [28] had speculated that PTU-induced AAV was self-limited and that eventually all immunosuppressive therapy could be discontinued. Our current study supported this speculation. In our study, all the patients with organ-specific involvement, except two patients with renal transplantation could discontinue immunosuppressive therapy within 12 months successfully; more importantly, no relapse was observed during subsequent follow-up of 45.1 months. Our experience suggested that the duration of immunosuppressive therapy in patients with PTU-induced vasculitis could be much shorter than those with primary AAV, as long as PTU was withdrawn, and further maintenance therapy might not be necessary in PTU-induced AAV. In fact, we have another four patients with PTU-induced AAV with renal involvement (data were not shown). After withdrawal of PTU and immunosuppressive therapy for 6–12 months, they all remained complete remission during follow-up for 12–24 months.

Although ANCA is an important serological marker for certain small vessel vasculitides, it might not be suitable for monitoring the disease activity of PTU-induced AAV. After discontinuation of PTU, even after immunosuppressive therapy, only 26.7% sera of our patients with PTU-induced AAV turned to MPO-ANCA negative despite clinical quiescence during follow-up. Actually, serum ANCA still remained positive in majority of our patients in complete remission for a long time up to 5 years. It has been reported that ANCA remained positive for >30 months in five children with PTU-induced AAV, and it did not turn to negative during follow-up of ~100 months in one patient [30]. Our previous study had shown that although the levels of MPO-ANCA decreased slowly, the other immunological characteristics of MPO-ANCA might change substantially. For example, the avidity of MPO-ANCA could decrease rapidly during follow-up, indicating that the avidity of anti-MPO antibody could be used as a more sensitive serological biomarker to reflect disease activity [31]. Furthermore, the levels of IgG4 subclass of MPO-ANCA decreased dramatically after cessation of PTU, indicating that the production of PTU-induced MPO-ANCA might be a result of chronic antigen (PTU) stimulation [25]. Other laboratory parameters such as ESR, which was associated with Birmingham vasculitis activity score in our previous study [25], might be better indicators for disease activity than the titres of ANCA.

During follow-up, microscopic haematuria remained positive in one patient with partial remission, though other laboratory parameters were all in normal range. It might be due to an underlying renal disease. Immune complex was found in renal histopathology in five out of the nine patients with renal biopsy in our study, which indicated that PTU-induced AAV may co-exist with other common primary renal disease [23].

For the treatment of hyperthyroidism, as most patients had received PTU therapy over 24 months, which was longer than recommendation in routine practice of anti-thyroid drug in Graves’ disease [32], it might indicate more severe immunological disorders of underlying thyroid disease in these patients. We recommend that the treatments such as subtotal thyroidectomy or radioactive 131I should be taken into consideration in further therapy for those with long-term PTU treatment. PTU and MMI are thioureylene derivatives, although possible cross-sensitivity due to structural similarities had been suggested and there were also several reports, in the literatures, on MMI-induced AAV, no relapse of vasculitis had been observed in the current study after switching PTU to MMI. Therefore, we recommended that MMI might be used as a substitution of PTU if necessary in those refusing to surgical intervention and radiation.

In our patients, the systemic symptoms were always ahead of or simultaneously with severe organ involvement, and patients with severe renal insufficiency could not achieve complete remission if PTU was not withdrawn in time. Based on these findings, we recommended that patients with PTU treatment should be monitored carefully during follow-up, especially those taking PTU for >2 yrs in order to diagnose PTU-induced AAV earlier. PTU should be discontinued immediately after diagnosis of PTU-induced AAV and appropriate immunosuppressive therapy according to disease severity, should be administered only for patients with vital organ involvement, such as lung and kidney vasculitis, in order to prevent progression to severe, irreversible disease. Furthermore, the immunosuppressive therapy might only last for 6–12 months without further maintenance therapy.

### Rheumatology key messages
- Different therapeutic strategy in PTU-induced AAV should be considered.
- Immunosuppressive therapy may be administered only for patients with vital organ involvement for 6–12 months.
- Maintenance therapy might not be necessary.

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