Outcome of anti-PL12 positive patients with antisynthetase syndrome

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Résumé

Profil évolutif du syndrome des antisynthétases avec anticorps anti-PL12

Objectifs > Le but de notre étude a été d’analyser la signification clinique et évolutive des anticorps anti-PL12 chez les patients ayant un syndrome des antisynthétases (SAS).

Méthodes > Les dossiers médicaux des patients ayant un SAS avec anticorps anti-PL12 (n = 5) ont été analysés. Afin d’exclure les faux-positifs, seuls les patients qui avaient une recherche positive d’anticorps anti-PL12 sur au moins deux prélèvements (en immunodot et/ou Western blot) ont été inclus dans cette étude.

Résultats > Les cinq patients avec anticorps anti-PL12 présentaient : une myosite (n = 2), un phénomène de Raynaud (n = 2), des mains du mécanicien (n = 1), des atteintes articulaires (n = 4), des localisations digestives (n = 2) et une pneumopathie interstitielle diffuse (PID) (n = 4). Les deux patients, atteints de myosite, ont eu une aggravation des manifestations musculaires sous traitement corticoides et immunosuppresseurs. De plus, les patients ont

Summary

Objectives > The aim of the present study was to assess the outcome in anti-PL12 patients with antisynthetase syndrome (ASS).

Methods > The medical records of anti-PL12 (n = 5) patients with ASS were retrospectively analyzed without prior selection. To exclude false-positive patients, we included patients who were successively tested positive for anti-PL12 antibody at least twice by immunodot and/or Western blot.

Results > Anti-PL12 patients experienced: myositis (n = 2), Raynaud’s phenomenon (n = 2), mechanic’s hands (n = 1), joint impairment (n = 4), digestive involvement (n = 2), and interstitial lung disease (ILD) (n = 4). The two patients with myositis exhibited deterioration of muscle manifestations despite therapy. As regards outcome of ILD, patients developed resolution (n = 1), stabilization (n = 1) or deterioration (n = 2) of pulmonary status. One patient died of pyogenic pneumonia.

Conclusion > Our series underscores that the presence of anti-PL12 antibody is associated with a particular phenotype of ASS characterized by: (1) less frequent although severe/steroid refractory myositis; (2) less common mechanic’s hands and
développé une guérison (n = 1), une amélioration (n = 1) ou une aggravation (n = 2) de la PID. Enfin, un patient est décédé de pneumonie infectieuse.

Conclusion > Notre série indique que les patients ayant un anticorps anti-PL12 ovaient un phénomène particulier de SAS caractérisé par : (1) une fréquence moins importante : (i) de myosite, mais qui semble grave et résistante aux traitements corticoides/immunosuppresseurs et (ii) d’un aspect de « mains du mécanicien » et de calcinoïse sous-cutanée ; et (2) une PID fréquente et sévère. Les patients atteints de PM/DM devraient avoir une recherche systématique d’anticorps anti-PL12, car cet auto-anticorps a un intérêt pronostique. En outre, un SAS sous-jacent devrait être recherché chez les patients porteurs de PID d’allure idiopathique associée à la présence d’anticorps anti-PL12.

Antisynthetase syndrome (ASS) is characterized by polymyositis/dermatomyositis (PM/DM) associated with antisynthetase antibodies, fever, arthritis, Raynaud’s phenomenon, mechanic’s hands and interstitial lung disease (ILD) [1–3]. Eight antisynthetase antibodies have been identified in patients with ASS, including anti-αSarc (anti-PL12) synthetase [3]. Anti-Jo1 antibody is the more common of antisynthetase antibodies [3,4]. In a series of 22 patients with antisynthetase syndrome, Koenig et al. [4] found anti-Jo1 antibody in 70% of cases; anti-PL12 antibody was more rare, accounting for 4.5% of antisynthetase antibodies. Only few series have analyzed the outcome in anti-PL12 patients with ASS. The aim of the current study was to: assess the outcome, including organ complications, functional course and mortality causes, in anti-PL12 patients with ASS.

Methods
The medical records of all anti-PL12 patients, in three university hospitals (Poitiers, Rennes, Rouen), were retrospectively analyzed without prior selection. The search for anti-PL12 antibody was performed by immunodot (immuno-DOT D-tek; Diasorin, Antony, France) and Western blot using protein extracts from Hep 2 cells. To exclude false-positive patients, we included patients who were successively tested positive for anti-PL12 antibody at least twice by immunodot and/or Western blot.

Initial evaluation of patients
Clinical data were obtained by a retrospective review of medical records. Anti-PL7 patients had an initial evaluation of organ involvement, including: (1) esophageal dysfunction. The diagnosis of ASS-related esophageal involvement was based on the presence of clinical manifestations: dysphagia, gastro-esophageal reflux into pharynx and/or mouth, coughing while eating, aphagia for solids and liquids [5]; (2) ASS-related gastrointestinal manifestations: hemorrhage related to vasculitis and chronic intestinal pseudo-obstruction; (3) respiratory muscle involvement resulting from ventilatory insufficiency related to striated muscle weakness; and (4) cardiac dysfunction (using electrocardiogram and echocardiography). In addition, patients were also examined for underlying malignancy.

Evaluation of interstitial lung disease (ILD)
Pulmonary involvement was investigated in all five anti-PL12 patients by pulmonary function tests (PFT) and high resolution computed tomography (HRCT-scan) of the lungs. Patients were classified subsequently into three groups according to presentation: (1) symptomatic acute onset of ILD; (2) symptomatic progressive onset of ILD; and (3) asymptomatic form with abnormalities consistent with ILD on PFT and HRCT-scan [6]. The following parameters were assessed: vital capacity (VC), forced vital capacity (FVC) and diffusing capacity of carbon monoxide (DLCO). VC and FVC were measured by spirometry (using a water-sealed spirometer); the DLCO was obtained by the single-breath method. Data were expressed as percentages of predicted values; the predicted values for each subject, based on sex, age, height, and weight, were obtained from standard tables [7]. Lung function was considered abnormal when volumes were less than 80% of predicted values and when DLCO was less than 70% of the predicted value. HRCT-scan was performed to evaluate radiographic abnormalities consistent with ILD, as described previously [3,6,8]. Severity of ILD on HRCT was scored according to Warrick et al. [3,6,8]. HRCT-scan pattern has been correlated with pulmonary histological findings, i.e.: (1) cryptogenic organizing pneumonia (COP); (2) nonspecific interstitial pneumonia (NSIP); (3) usual interstitial pneumonia (UIP); and (4) diffuse alveolar damage (DAD) [3,6,8]. Our patients were, thus, divided into four groups based...
on the predominant pattern on HRCT: COP, NSIP, UIP and DAD [3,6,8]. Finally, one patient underwent transbronchial or surgical lung biopsy at ILD diagnosis; histologic analysis of lung biopsy specimens was made to detect abnormalities consistent with ILD: COP, NSIP, UIP or DAD.

**Outcome of patients**

All anti-PL12 patients received specific therapy. Data were reviewed for therapy used at any time during the course of PM/DM: steroids (prednisone, pulsed intravenous doses of methylprednisolone), cytotoxic drugs (methotrexate, azathio- prine, cyclophosphamide, ciclosporin, mycophenolate mofetil), as well as intravenous immunoglobulins. Patients had a minimal follow-up of 18 months, although patients who died before the 18-month follow-up were also included. The outcome of patients was defined as: (1) remission: stable increase of muscle strength and normalization of serum muscle enzyme levels (CK) as well as disappearance of skin manifestations, persisting after therapy discontinuation; (2) improvement: when muscle and skin signs improved and CK levels decreased with therapy; and (3) deterioration when muscle and skin signs worsened, and CK levels increased despite therapy. Recurrences of ASS were diagnosed on the basis of clinical relapses. Recurrences were divided into short- and long-term recurrences: (1) short-term recurrences occurred during tapering of therapy; and (2) long-term recurrences occurred after termination of therapy. Survival status was based on hospital records; the causes of death were determined through hospital or physician records.

**Literature review**

We recorded the English-language literature in the PubMed database for related articles published up to September 2012, using the following keywords: “PL12 antibody”‘; “anti-PL12’’; “anti-alanyl-tRNA synthetase” and “antisynthetase syndrome”.

**Results**

Five consecutive ASS patients with anti-PL12 antibody were included in the study. All patients were seen at university medical centers as inpatients or outpatients between 1996 and 2011. All patients exhibited one or more symptoms of ASS symptoms, including ILD and/or myositis. None of these patients with ASS had other connective tissue disorders, especially systemic lupus erythematosus, systemic sclerosis and Sjögren’s syndrome.

**General characteristics of anti-PL12 patients**

The five anti-PL12 patients consisted of one man and four women with a median age of 52 years [range: 31–65] at ASS diagnosis. Four patients had PM and one had DM. Patients developed ASS-related complications: Raynaud’s phenomenon (n = 2), mechanic’s hands (n = 1), esophageal dysfunction (n = 1) and hemorrhage related to gastrointestinal vasculitis (n = 1) (table I). Four patients had joint manifestations, i.e.: arthralgia (n = 4), erosive (n = 1)/non-erosive (n = 3) arthritis.

**Characteristics of ILD in anti-PL12 patients**

Four anti-PL12 patients had ILD. ILD onset preceded other ASS signs in one patient, and was concurrently identified in association with ASS in three patients. At ILD diagnosis, pulmonary symptoms consisted of: dyspnea (n = 3) and cough (n = 1). Patients were divided into three groups according to their presenting lung manifestations: (1) symptomatic acute onset of ILD (n = 1); (2) symptomatic progressive onset of ILD (n = 2); and (3) asymptomatic patients exhibiting abnormalities consistent with ILD on PFT and HRCT of the lungs (n = 1). At ILD diagnosis, the median values of PFT parameters were: 77% for FVC, 73% for VC and 45% for DLCO. HRCT-scan of the lungs demonstrated: parenchymal micronodules/nodules (25%), linear opacities (75%), irregularity of the interfaces (100%), ground-glass opacities (100%), honeycombing (25%), consolidation (25%) and traction bronchiectases/bronchiolectases (25%). In our patients, the median score of fibrosis on HRCT-scan was 20.3. Based on HRCT-scan pattern, patients were divided into the following groups: COP (n = 1) and NSIP (n = 3). One patient underwent transbronchial lung biopsy; histologic analysis of pulmonary biopsy specimens demonstrated damage consistent with NSIP. In this patient, we found a relationship between HRCT-scan pattern and histologic pulmonary damage.

**Course of anti-PL12 patients**

The median follow-up duration of anti-PL12 patients was 46 months [range: 5–84 months]; none of the patients was lost to follow-up. All patients were given steroid therapy (1 mg/kg/day); because of steroid-refractory ASS, patients were additionally treated with: methotrexate (n = 2) and azathioprine (n = 1). The outcome of these patients is shown in table I.

- **outcome of myositis:** the two patients with muscle involvement exhibited deterioration of myositis at last follow-up;
- **outcome of joint involvement:** in our four patients with joint signs, the outcome was as follows: remission (n = 1), improvement (n = 1) and deterioration (n = 2);
- **outcome of ILD:** at last follow-up, the median values of PFT parameters were: 74% for FVC, 71% for VC and 41% for DLCO. In two anti-PL12 patients, ILD resolved (n = 1) or stabilized (n = 1); these two patients had symptomatic progressive onset of ILD (n = 1) and asymptomatic form (n = 1). In the two remaining patients, ILD worsened despite therapy; these two patients exhibited acute onset of ILD (n = 1) and symptomatic progressive onset of ILD (n = 1).

Finally, one patient died; death was due to pyogenic pneumonia at 5 months follow-up in this patient who exhibited acute onset of ILD.
In our literature review of anti-PL12 patients [1,9–18], we only identified 77 well-described cases of anti-PL12 patients, most of which were case reports [1,9–12,14,15,17,18]. The prevalence of clinical features in anti-PL12 patients is shown in Table I.

**Table I**

<table>
<thead>
<tr>
<th>Characteristics of anti-PL12 positive patients with ASS¹</th>
<th>Present series (n = 5)</th>
<th>Previous reports¹ (n = 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>Males (n = 1)/females (n = 4)</td>
<td>25% males (n = 19)/75% females (n = 58)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>52</td>
<td>45</td>
</tr>
<tr>
<td><strong>Subset of myositis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM¹</td>
<td>(n = 4)</td>
<td>76% (n = 59)</td>
</tr>
<tr>
<td>DM¹</td>
<td>(n = 1)</td>
<td>24% (n = 18)</td>
</tr>
<tr>
<td><strong>Systemic manifestations of PM/DM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myositis (myalgia and/or muscle weakness)</td>
<td>(n = 2)</td>
<td>51% (n = 39)</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>(n = 2)</td>
<td>59% (n = 45)</td>
</tr>
<tr>
<td>Mechanic’s hands</td>
<td>(n = 1)</td>
<td>37% (n = 28)</td>
</tr>
<tr>
<td>Calcinosis cutis</td>
<td>(n = 0)</td>
<td>NA¹</td>
</tr>
<tr>
<td>Esophageal involvement</td>
<td>(n = 1)</td>
<td>24% (n = 18)</td>
</tr>
<tr>
<td>Gastrointestinal involvement</td>
<td>(n = 1)</td>
<td>NA¹</td>
</tr>
<tr>
<td>Arthralgia/arthritis</td>
<td>(n = 4)</td>
<td>47% (n = 36)</td>
</tr>
<tr>
<td>ILD¹</td>
<td>(n = 4)</td>
<td>97% (n = 75)</td>
</tr>
<tr>
<td>Pyogenic pneumonia</td>
<td>(n = 1)</td>
<td>NA¹</td>
</tr>
<tr>
<td>Ventilatory insufficiency related to striated muscle weakness</td>
<td>(n = 1)</td>
<td>0% (n = 0)</td>
</tr>
<tr>
<td><strong>Cancer</strong></td>
<td>(n = 0)</td>
<td>2.5% (n = 2)</td>
</tr>
<tr>
<td><strong>Outcome of myositis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>(n = 0)</td>
<td>NA¹</td>
</tr>
<tr>
<td>Improvement</td>
<td>(n = 0)</td>
<td>NA¹</td>
</tr>
<tr>
<td>Deterioration</td>
<td>(n = 2)</td>
<td>NA¹</td>
</tr>
<tr>
<td><strong>Outcome of ILD¹</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>(n = 1)</td>
<td>NA¹</td>
</tr>
<tr>
<td>Improvement</td>
<td>(n = 1)</td>
<td>NA¹</td>
</tr>
<tr>
<td>Deterioration</td>
<td>(n = 2)</td>
<td>NA¹</td>
</tr>
<tr>
<td>Mortality</td>
<td>(n = 1)</td>
<td>9% (n = 7)</td>
</tr>
</tbody>
</table>

ASS: antisynthetase syndrome; PM/DM: polymyositis/dermatomyositis; ILD: interstitial lung disease; NA: not available; except where indicated, values are median.

¹Identified in Medline search, references [1,9–12,15,17,18].

**Literature review**

In our literature review of anti-PL12 patients [1,9–18], we only identified 77 well-described cases of anti-PL12 patients, most of which were case reports [1,9–12,14,15,17,18]. The prevalence of clinical features in anti-PL12 patients is shown in Table I.

**Discussion**

The antisynthetase antibodies are the most widely recognized myositis specific autoantibodies [19,20]. The PL12 antigen is a 107,958 dalton polypeptide that coprecipitates with tRNA; anti-PL12 antibody was first described in 1986 by Bunn and coworkers [9], and it is directed against...
alanyl-tRNA synthetase. Anti-PL12 antibody has been reported in 5–10% of patients with ASS and in less than 2% of overall patients with PM/DM [2,3,19–24].

To date, whereas the characteristics of anti-Jo1 patients are well known, the outcome of anti-PL12 patients has been uncommonly evaluated. Furthermore, previous series have mainly included anti-PL12 patients with overlap syndrome of ASS with systemic sclerosis and/or Sjögren’s syndrome [13]; altogether, no definite conclusion can be drawn from previous data regarding the accurate features of anti-PL12 patients with isolated ASS. The current series has, in fact, considered anti-PL12 patients with isolated ASS without prior selection based on clinical presentation.

In our literature search, using the Medline database (1966–2012), we have identified 77 well-described cases of anti-PL12 patients with ASS [1,9–12,14,15,17,18]. The average age of these patients at the time of ASS diagnosis was 45 years (range: 22–87 years); 75% of patients were women [1,9–12,14,15,17,18]. The current study shows that most anti-PL12 patients were women with a median age of 52 years.

Muscle involvement can postdate other manifestations in anti-Jo1 patients, especially ILD, although myositis may lack [1–3,6,8]. In our review of 77 anti-PL12 ASS, 51% of patients experienced myositis [1,9–12,14,15,17,18]. In this instance, 40% of our anti-PL12 patients exhibited clinical myositis. Our findings show that the presence of anti-PL12 antibody may be associated with a particular phenotype of ASS – that is amyopathic ASS. To date, the outcome of myositis is still unknown in anti-PL12 patients; only one series has reported that 60% of patients had improvement/stabilization of myositis, whereas 40% of patients exhibited deterioration of muscle signs [11]. In our experience, the presence of anti-PL12 antibody was strongly associated with worsening of muscle manifestations despite appropriate therapy in these patients. Interestingly, our data indicate that the presence of anti-PL12 antibody confers a poor outcome of myositis in ASS. Additionally, in our review of 77 anti-PL12 patients, joint manifestations were present in 47% of cases [1,9–12,14,15,17,18]. The current study shows a higher prevalence of joint involvement, arthralgia/arthritis being encountered in 80% of anti-PL12 patients. Furthermore, half of our patients exhibited deterioration of joint manifestations (determined by increased count of painful and/or swollen joints); none of these patients exhibited concurrently: rheumatoid factors, anti-CCP and/or anti-Ro52 antibody. Thus, our study underscores that the presence of anti-PL12 antibody seems to be associated with both frequent and severe joint involvement in ASS.

Anti-Jo1 antibody is a strong predictive factor for developing ILD in the setting of PM/DM [2,3,6,8]. In a series of 88 Japanese patients with isolated ILD and antisynthetase antibodies, anti-PL12 antibody has been depicted in up to 14% of cases [18]. In our review of 77 anti-PL12 ASS, 97% of patients exhibited ILD [1,9–12,14,15,17,18]. The present study reinforces the marked association between ILD and the presence of anti-PL12 antibody as 80% of our patients experienced ILD; ILD was more often identified concurrently in association with ASS, although it preceded ASS onset in other patients. Taken together, our findings underscore that: (1) patients with ILD should systematically undergo the search for anti-PL12 antibody for predicting underlying ASS; and (2) anti-PL12 patients should routinely undergo ILD screening (through PFTs and HRCT-scan), resulting in both earlier diagnosis and management of ILD. Regarding lung presenting manifestations, we have further found that anti-PL12 patients uncommonly exhibited asymptomatic form of ILD, whereas most patients developed symptomatic acute/progressive onset of ILD. Our series interestingly showed that anti-PL12 antibody was associated with a large spectrum of ILD, including COP and NSIP, although NSIP was the most frequent pattern (75% of cases). Our study was, to our knowledge, the first to evaluate the outcome of ILD in anti-PL12 patients. We have found that 25% of anti-PL12 patients had worsening of pulmonary status, whereas other patients exhibited either improvement (50%) or remission (25%) of ILD. Our data suggest that anti-PL12 patients may exhibit a less severe form of ILD than anti-Jo1 ASS patients (8); the optimal therapy remains unclear in such patients [25].

We have analyzed other extramuscular features of anti-PL12 patients with ASS. In our review of 77 anti-PL12 patients, Raynaud’s phenomenon and mechanic’s hands were found in 59% and 37% of cases, respectively [1,9–12,14,15,17,18]. Our study has shown that anti-PL12 patients uncommonly exhibited mechanic’s hands (20% of cases) and calcinosis cutis (0%). Altogether, our findings suggest that anti-PL12 patients develop a particular cutaneous phenotype of ASS characterized by: lower frequency of mechanic’s hands and absence of calcinosis cutis. Regarding digestive tract involvement, we have found that only 24% of the 77 anti-PL12 patients had esophageal involvement [1,9–12,14,15,17,18]. In this instance, 20% of anti-PL12 patients developed esophageal dysfunction; another patient exhibited hemorrhage related to gastrointestinal vasculitis. Our study showed that digestive complications should not be overlooked in this subgroup of patients.

In conclusion, our series underscores that the presence of anti-PL12 antibody is associated with a particular phenotype of ASS characterized by: (1) less frequent although severe/steroid refractory myositis; (2) less common mechanic’s hands and calcinosis cutis; (3) both frequent and severe ILD. Taken together, our findings have shown that PM/DM patients should routinely undergo the search for anti-PL12 antibody as this autoantibody appears to impact patients’ prognosis. Furthermore, ILD patients with anti-PL12 antibody should routinely undergo clinical screening for underlying ASS.

Disclosure of interest: the authors declare that they have no conflicts of interest concerning this article.
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References


