Clinical significance of anti-Ro/SSA-52 kDa antibodies—a retrospective monocentric study

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Introduction

Two types of anti-Ro/SSA antibodies have been identified. They are specific for two different Ro/SSA antigens of 60 kDa and 52 kDa [1, 2] derived from the RNP complex. Anti-SSA-60 kDa antibodies (aSSA60) are linked to certain disorders such as SS, SLE, neonatal lupus and congenital heart block [2–5]. Even though anti-SSA-52 kDa antibodies (aSSA52) have recently been implicated in the occurrence of congenital heart block [6–9], controversies still exist regarding the clinical relevance of aSSA52 in adult autoimmune diseases (ADs).

The SSA-52 antigen is bound to the RNP complex via protein–protein interactions [10] and details of this binding are not well understood. Moreover, it has been shown that aSSA52 recognizes linear epitopes of the protein that would be hidden when the protein is folded into its tertiary structure [11–14]. There might also be a non-specific interaction between a terminal domain of the SSA-52 protein and the hinge region of the immunoglobulin.

Clinically, the presence of aSSA52 has been reported in a wide variety of diseases. The spectrum of autoimmune disorders associated with aSSA52 antibodies is broader than that associated with aSSA60 antibodies, and includes inflammatory myositis [15], primary biliary cirrhosis [16] and SSc [17, 18]. aSSA52 antibodies also tend to be associated with non-ADs such as viral infections [18–20] or neoplastic diseases [21]. Moreover, the prevalence of these antibodies in the general population is not negligible [22].

In order to determine the clinical relevance of these antibodies, we investigated the specificity of aSSA52 antibodies in ADs. We conducted a retrospective, monocentric study of all patients with known anti-Ro/SSA antibody statuses over a 3-year period. We investigated the clinical features of patients who were aSSA52 positive (aSSA52+) and aSSA60 negative (aSSA60−).

Patients and methods

Inclusion criteria

All patients who had been screened for aSSA52 and were listed in the database of the immunology laboratory of our University Hospital in Nantes between 1 January 2005 and 1 March 2008 were investigated. Only the patients who were aSSA52+ (≥ 40 UI) and aSSA60− (< 40 UI) were included in the study. Three patients who met these criteria were excluded: two because of missing files and one patient with neonatal lupus, whose antibodies were similar to his mother’s, who was already included in the study. Ethics approval for the study was given by the clinical research group of our hospital.

Quantitative and qualitative data on the autoimmune status of our cohort were compared with those of a control group of patients who were aSSA52+/aSSA60+ and aSSA52−/aSSA60+ (Fig. 1). Data for the control group consisted of patients tested for autoantibodies at our institute during the same period.

Definition of autoimmunity

A patient was defined as having an AD when he displayed one of these: SSc, autoimmune cytopenia, myositis with or without anti-synthetase antibodies, SLE, chronic cutaneous lupus, RA, SS, AS, TA, PMR, autoimmune hepatitis or cryoglobulinaemia. All in accordance with the international criteria for classification (i.e. 1997 ACR criteria for SLE [23], 2002 Americano–European Consensus for SS [24]). As this was a retrospective study, we did not have complete information concerning pregnancy events, and were thus unable to evaluate the occurrence of congenital heart block.

Definition of the principal diagnosis

The principal diagnosis was either that for which the patient had been followed for a long period of time, or that for which a specific treatment was in process. In the case of multiple associated diseases, the main disease was considered to be the one for which the patient was undergoing specific care at the time of antibody testing.

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Autoantibodies were detected by IF analysis using HEP2 cells (Bio-Rad, Marnes-La-Coquette, France). The fluorescence pattern and antibody titre were assessed by two different technicians.

Antibody titres
Screening for and titration of antibodies against ANAs and anti-ENAs were performed using the Lumines/FIDIS technique (Connective 10, BMD, France) [25]. This test was conducted only when the IF indicated a titre ≥1/160, or at the specific request of the physician (seven cases). When several titrations were made for a single patient, we used the oldest one, or the one displaying the greater number of antibodies with different specificities.

Definition of isolated aSSA52
We considered aSSA52/aSSA60 isolated specimens to be those where aSSA52 and/or aSSA60 were positive, but other autoantibodies [ANAs, soluble antigen antibodies (other than aSSA), anti-tissue antibodies (ASMAs, anti-actin or AMAs), anti-cytoplasmic antibodies, and a positive Coombs test] were negative.

Statistical analysis
Statistical analysis was performed using GRAPHPAD software. Mann–Whitney tests were used to compare means of two qualitative variables and Fisher’s and/or chi-square tests were used to compare means of two quantitative variables.

Results
Prevalence of autoimmunity
Eighty-two patients were included in the study. There were 21 males and 61 females (sex ratio, 0.34). The average age of patients at the time of testing was 57 (range 3–97) years. The medium follow-up time after immunological testing was 6.5 (range 0–31) months.

The prevalence of ADs in the whole cohort of aSSA52+/aSSA60− patients was 58.5% (48 patients). This frequency was significantly lower in aSSA52+/aSSA60− patients compared with aSSA52+/aSSA60+ patients (79.5%, P = 0.001) and to aSSA52−/aSSA60+ patients (77.8%, P = 0.02) (Table 1).

We divided the aSSA52+/aSSA60− patients into four groups (Fig. 1): Group 1 included 34 aSSA52+/aSSA60− patients who also expressed other autoantibodies. Among these, 33 patients (Group 1a) had an autoimmune disorder (97.1%). The remaining patients had undergone allogeneic bone marrow transplantation for acute lymphoblastic leukaemia (Group 1b).

Group 2 included 48 aSSA52+/aSSA60− patients who did not express other autoantibodies. Fifteen (31.3%) of these had an AD (Group 2a), whereas 33 patients had no AD (Group 2b).

Group 1
Quantitative data. The prevalence of autoimmunity in this group was high (97.1%) and comparable with that in the two groups of patients aSSA52+/aSSA60+ or aSSA52−/aSSA60+.

Qualitative data. Thirteen different antibodies were associated with aSSA52 with variable frequencies (Table 2). Ten different ADs were recorded, with SSc (eight cases) and SLE (nine cases) being the commonest.

We assessed the diagnostic value of aSSA52. We examined specific clinical features of the commonest ADs. Regarding SLE, the frequency of lupus nephropathy was low (one case), but this was not statistically different from that for SLE in aSSA60+ patients (11 cases of nephropathy out of 31) (P = 0.23). SSc cases were always associated with ACAs (eight cases). There were five cases of lcSSc, two cases of dSSc, and one case of Reynolds syndrome (associated with primitive biliary cirrhosis).

Group 2
Quantitative data. The prevalence of AD in this group was low (31.3%) and was significantly lower compared with aSSA52+/aSSA60+ patients without other autoantibodies (88.8%, P = 0.003) or to aSSA52−/aSSA60+ patients without other autoantibodies (88.9%, P = 0.01).

Qualitative data. Eight different ADs were identified in Group 2a patients (Table 3). Chronic viral diseases (six cases of either HBV, HCV or HIV), and active or in remission neoplastic disease (eight cases) were frequently encountered in Group 2b patients. Seven of these patients (21.2% of Group 2b patients) were receiving treatment with IFN-α, which is known to induce autoimmunity. Five patients from Group 2b were receiving β-blockers.
Clinical value of aSSA52

The presence of isolated aSSA52 was not sufficient to predict the presence of an AD. When associated with other antibodies, aSSA52 showed no value for the positive or differential diagnosis of ADs. Our study focused on SLE and SSc, and the presence of aSSA52 was not associated with any particular clinical symptoms, to the contrary of what had been suggested during other autoimmune diseases such as anti-synthetase syndrome [29] and primary biliary cirrhosis [16]. No specific clinical or biological features of SLE were associated with the presence of isolated aSSA52. The proportion of lupus nephritis tended to be lower in comparison with aSSA52+/aSSA60+ and aSSA52−/aSSA60+ SLE patients, but this difference was not statistically significant. In addition, the presence of aSSA52 was not protective, since nephritis was as common in aSSA52-positive patients as in aSSA60-positive patients.

In the case of SSc, ACAs were always present, but anti-scl-70 antibodies were never present. The clinical phenotypes corresponded to the current epidemiology of the disease. Our series was unable to demonstrate a link between antibody status and any specific clinical anomaly in other ADs.

The results of our study showed that the presence of aSSA52 was of no diagnostic value in patients presenting with ADs, and that other co-expressed autoantibodies had better diagnostic discriminatory value.

The role of IFN-α in aSSA52 generation

IFN-α is a key cytokine in different ADs, including SLE [30, 31], and acts as a non-specific immune system activator. Exogenous IFN-α is also known to induce autoimmunity [32, 33], especially ANAs. Interestingly, seven (21.2%) aSSA52-isolated patients were treated with IFN-α. However, all of them were belonging to Group 2b, which means they were clinically asymptomatic. The role of IFN-α in aSSA52 emergence is difficult to determine herein, as all these patients were treated for infectious or neoplastic Diseases (also known to induce autoimmunity). As patients treated with IFN-α are closely monitored, the prevalence of that kind of patient could have been underestimated in our study. Moreover, the proportion of IFN-α-treated patients in our cohort does not represent the prevalence of aSSA during this treatment.

Discussion

Low prevalence of ADs in patients with aSSA52 antibodies

In our series, 58.5% of aSSA52+/aSSA60− patients had an associated AD. This proportion was smaller when the aSSA52+/aSSA60− pattern was not associated with any other autoantibodies (31.3%). This one-third prevalence was not negligible, and higher than in the general population [22]. Nevertheless, this proportion was significantly lower than in the groups of aSSA52+/aSSA60+ patients and of aSSA52−/aSSA60+ patients. Moreover, 40% of ADs diagnosed in the case of isolated aSSA52 were autoimmune cytopenia, AS and polymyalgia rheumatica, diseases specifically associated with anti-Ro/SSA autoantibodies.

With a lower prevalence of autoimmunity in our study than in previously published series [18, 26, 27], our result suggests that isolated aSSA52 was only slightly associated with the presence of ADs. It would be interesting to perform a second, later titration of aSSA52, to determine its persistence. Eleven patients in our cohort underwent longitudinal monitoring and two cases became antibody negative.

In ADs such as SLE, autoantibodies could be present before the occurrence of clinical symptoms [28]. Our series did not allow us to assess the longer-term risk of developing an AD in patients with isolated aSSA52 (average follow-up, 4.1 months), and prospective monitoring is required to evaluate this risk. Clinicians should be aware of this possibility, and should regularly monitor patients at follow-up. Indeed, recent data concerning occurrence of congenital heart block in aSSA52+ pregnant women [6–9] also impose a close follow-up in young women.

Table 2. Autoantibodies associated with aSSA52 in the aSSA52+/aSSA60− patients

<table>
<thead>
<tr>
<th>Autoantibodies associated with aSSA52</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACAs</td>
<td>13</td>
</tr>
<tr>
<td>Anti-DNA autoantibodies</td>
<td>10</td>
</tr>
<tr>
<td>Anti-SSB autoantibodies</td>
<td>4</td>
</tr>
<tr>
<td>anti-JO1 autoantibodies</td>
<td>3</td>
</tr>
<tr>
<td>aCCP autoantibodies</td>
<td>3</td>
</tr>
<tr>
<td>aSm autoantibodies</td>
<td>2</td>
</tr>
<tr>
<td>Combs</td>
<td>2</td>
</tr>
<tr>
<td>RF</td>
<td>4</td>
</tr>
<tr>
<td>Anti-RNP autoantibodies</td>
<td>2</td>
</tr>
<tr>
<td>AMAs</td>
<td>1</td>
</tr>
<tr>
<td>ASMAEs</td>
<td>1</td>
</tr>
<tr>
<td>Cryoglobulinaemia</td>
<td>1</td>
</tr>
<tr>
<td>Anti-ribosomal autoantibodies</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3. Occurrence of ADs in Group 2a

<table>
<thead>
<tr>
<th>ADs in Group 2a</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>4</td>
</tr>
<tr>
<td>Autoimmune cytopenia</td>
<td>3</td>
</tr>
<tr>
<td>AS</td>
<td>2</td>
</tr>
<tr>
<td>Chronic cutaneous lupus</td>
<td>2</td>
</tr>
<tr>
<td>SLE</td>
<td>1</td>
</tr>
<tr>
<td>SSc</td>
<td>1</td>
</tr>
<tr>
<td>Myositis, anti-synthetase syndrome</td>
<td>1</td>
</tr>
<tr>
<td>TA, PMR</td>
<td>1</td>
</tr>
</tbody>
</table>

AD could not be diagnosed according to our criteria in eight cases (undifferentiated rheumatism, six cases; isolated pulmonary arterial hypertension, two cases). The other patients had more common diseases (including hypertensive nephropathy and ischaemic cardiopathy).

The average titres of aSSA52 in Groups 2a and 2b were not significantly different: 135 ± 106 (P = 0.25).

Conclusion

In this retrospective study, which was not conducted to assess to the occurrence of congenital heart block, the presence of isolated aSSA52 is not consistently associated with autoimmune disorders, and its presence is weakly predictive of autoimmunity. However, in the absence of any neoplastic or viral diseases, or any treatment inducing ANAs, it could be useful to confirm the aSSA52 status during follow-up.

aSSA52 also had a low diagnostic and prognostic value when associated with other autoantibodies.

Further prospective studies are needed to assess the risk of developing ADs in aSSA52-positive patients, including congenital heart block.

Rheumatology key messages

- A new test for a new anti-Ro/SSA antibody (52 kDa) is available.
- This retrospective study of 297 patients is comparing the clinical significance of different antibodies: anti-Ro/SSA-52, anti-Ro/SSA-60 and anti-la/SSB antibodies.
- The clinical significance of anti-Ro/SSA-52 antibodies in terms of auto-immunity is limited.

Disclosure statement: The authors have declared no conflicts of interest.
Clinical significance of anti-Ro/SSA antibodies

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