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Proposed cut-off for reactivity of anti-HMGCR and anti-SRP antibodies in patients statin-exposed and statin-unexposed

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Abstract

The therapeutic approach with statins is widely used in the control of dyslipidemias. However, there is no laboratory evaluation to elect patients to make use of this class of therapeutic drugs.

We analyzed the prevalence of anti-signal recognition particle (anti-SRP) and anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR) antibodies in a heterogeneous cohort of 85 patients in order to determine cutoff reference values for these antibodies.

Serum samples from 85 patients were screened for the presence of anti-HMGCR and anti-SRP autoantibodies by enzyme-linked immunosorbent assay. The demographic, clinical, and morphological features were also correlated with anti-HMGCR and anti-SRP antibodies. The patients were divided in 2 groups: A, statin-exposed, and B, statin-unexposed.

There was no significant association ($P > .05$) among anti-HMGCR and anti-SRP titers in relation to age, sex, statin exposure, and CK level. The concentrations of both antibodies were not correlated with symptoms, CK level, or statin exposure. Eleven (12.9%) patients had anti-HMGCR antibodies. We found a tendency ($P = .051$) toward greater anti-HMGCR positivity in women with no symptoms. Twelve (14.1%) patients had anti-SRP antibodies. There was no sex predominance, and only 1 patient had muscle complaints. Muscular symptoms were present in 31 (36.5%) patients, 4 (12.9%) were positive for anti-HMGCR antibodies, and 1 (3.2%) was positive for anti-SRP antibodies. A total of 54 (63.5%) patients had no muscle symptoms, 7 (13%) were anti-HMGCR positive, and 11 (20.4%) were anti-SRP positive. We found statistical significance for patients with anti-SRP antibodies when asymptomatic and symptomatic patients were compared ($P = .029$). In contrast, there was no statistically significant difference between symptoms and positivity for anti-HMG antibodies.

One of the main aims of this study was to define a cutoff point in a heterogeneous population with different diagnoses. We also demonstrated that anti-HMGCR and anti-SRP antibodies are not 100% specific to immune-mediated necrotizing myopathy. We believe that these antibodies must be tested and interpreted within the specific context.

Abbreviations: CK = creatine kinase, HMGCoA = 3-hydroxy-3-methylglutaryl-coenzyme A, HMGCR = 3-hydroxy-3-methylglutaryl-coenzyme A reductase, IM = inflammatory myopathies, IMNM = immune-mediated necrotizing myopathy, kDA = kilodalton, LR = negative likelihood, LR+ = positive likelihood, ROC = receiver-operating curve, SRP = signal recognition particle.

Keywords: Anti-HMGCR antibody, anti-SRP antibody, HMGCoA reductase, immune-mediated necrotizing myopathy, statin-exposed

1. Introduction

In 1976, Japanese investigators presented good evidence that specific fungal metabolites were effective inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase; HMGCR), an enzyme which decreases cholesterol synthesis in rats, hens, and dogs without affecting any other enzymes involved in this pathway.[1] Later, they demonstrated that mevastatin, the prototype HMGCR inhibitor, also reduces serum cholesterol concentrations in humans with hypercholesterolemia. That drug was followed by another related drug called lovastatin, which drastically reduced cholesterol levels in normal subjects.[2] Since then, a new age of HMGCR inhibitors has led to important advances in the treatment of hypercholesterolemia.

Inhibitors of HMGCR act on a crucial step of cholesterol biosynthesis, the so-called "mevalonate pathway." They inhibit HMGCR, thereby reducing mevalonate synthesis. As a consequence, several other isoprenoid pathways are also affected including ubiquinone, which takes part in mitochondrial electron transport, dolichol, which is required for glycoprotein synthesis, and isopentenyl adenine.[3]

In 2010, Christopher-Stine et al identified a new autoantibody that recognizes 2 proteins, 200- and 100 kilodalton (kDa), related to a necrotizing myopathy that had not been previously identified. Interestingly, this antibody was found to have a

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particularly high prevalence in individuals who had been exposed to statins.[4,5]

In 2011, Mamen et al demonstrated that statin use upregulates expression of the ~200 and ~100-kDa autoantigens. In this report, they demonstrated a likely causal link between statin exposure and this distinct form of necrotizing myopathy through identification of the autoantigen as HMGCR. Immunoprecipitation assays demonstrated the specificity of the autoantibodies for the carboxyl terminus of this enzyme, whereas competition experiments confirmed that anti-HMGCR autoantibodies immunoprecipitated both HMGCR and the ~200-kDa protein. Since then, the necrotizing myopathy has been named immune-mediated necrotizing myopathy (IMNM), and is associated with anti-HMGCR.[6]

The signal recognition particle (SRP) is a cytoplasmic ribonucleoprotein that binds the signal sequences of newly synthesized proteins and facilitates their translocation into the endoplasmic reticulum. Recognition occurs as soon as the signal sequence has emerged from the ribosome and involves the 54-kDa protein of the SRP. In 1986, antibodies-recognizing components of the SRP were described for the first time in the serum of a patient with polymyositis.[6,7] Later, it was demonstrated that anti-SRP autoantibodies are associated with a necrotizing myopathy syndrome in the spectrum of immune-mediated myopathies that differ from typical polymyositis.[8]

In summary, anti-200/100 patients share certain features with the anti-SRP population; however, these antibodies represent 2 immunologically distinct groups as the anti-200/100 sera did not recognize any of the SRP subunits. In addition, anti-SRP sera did not recognize proteins with molecular weights of 200 or 100 kDa.[4]

Based on this, we analyzed the prevalence of anti-SRP and anti-HMGCR antibodies in a heterogeneous cohort of 85 patients to determine cutoff reference values for these antibodies. The therapeutic approach with statins is widely used in the control of dyslipidemias. However, there is no laboratory evaluation to elect patients to make use of this class of therapeutic drugs.

2. Methods

A total of 85 serum samples were collected from patients who attended an outpatient clinic from School of medicine of ABC. These samples were screened for the presence of anti-HMGCR and anti-SRP autoantibodies by enzyme-linked immunosorbent assay (ELISA; CUSABIO kit).

We selected 3 groups of patients: those with muscle complaints (myalgia, fatigue, cramps, weakness, and dysphagia) and/or elevated creatine kinase (CK) levels, who had or had not been exposed to statins, and had undergone muscle biopsy; patients who had been exposed to statins but did not have neuromuscular symptoms; and a control group made up of healthy individuals.

We analyzed demographic data (age and sex), CK levels, muscle complaints, and morphological muscle findings (when a muscle biopsy was performed) to find correlations between them and the results for anti-HMGCR and anti-SRP antibody levels.

Finally, we divided the subjects into 2 groups based on statin exposure: statin-exposed (group A) and statin-unexposed (group B).

The review board approved this study, and written informed consent was obtained from each participant.

2.1. Statistical analysis

Qualitative variables were described by absolute and relative frequencies and quantitative variables by median and 25% and 75% percentiles. To identify the best cutoff point for the antibodies studied for determination of the positive values, we used receiver-operating curve (ROC) analysis to estimate the sensitivity, specificity, accuracy, positive likelihood (LR+), and negative likelihood (LR–).

We then assessed the concentration of antibodies, which were classified as either reagent or no reagent. To analyze the association between antibody titers and clinical characteristics including age, sex, statin exposure and biopsy findings, the χ^2 test was used. However, the Mann-Whitney test was used to analyze differences between age and CK levels according to antibody titers because the quantitative variables were abnormal according to the Shapiro-Wilk test ($P < .05$).

The confidence level adopted was 95%. The statistical software used was Data Analysis and Statistical Software for Professionals (Stata) version 11.0.

3. Results

The predictive capacity of anti-HMGCR and anti-SRP antibodies for predicting statin use was 0.557 (area under ROC curve of 0.557; 95% confidence interval [CI] [0.426, 0.688]) and 0.390 (area under ROC curve of 0.391; 95% CI [0.254, 0.527]), respectively.

The sensitivity, specificity, accuracy, LR+ and LR– for the various cut-off points of the anti-HMGCR and anti-SRP antibodies are shown in annex 1. The cut-off points that had the best values of sensitivity, specificity, accuracy, LR+ and LR– of anti-HMGCR and anti-SRP antibodies for predicting statin use were 2.283 and 0.348, respectively. The cut-off point of 2.283 for anti-HMGCR showed a specificity of 85.71%, sensitivity of 14.4%, accuracy of 63.53%, LR+ of 0.983 and LR– of 1.00339. The cut-off point of 0.348 for anti-SRP showed a specificity of 78.57%, sensitivity of 12.28%, accuracy of 62.35%, LR+ of 0.5736 and LR– of 1.116.

Our sample of 85 patients was heterogeneous. Of the 85 patients, 33 had undergone a muscle biopsy and had been exposed to statins, 14 had a muscle biopsy but had not been exposed to statins, 24 were exposed to statins but had no muscular symptoms, and 14 were healthy patients.

There was no significant association ($P > 0.05$) among anti-HMGCR and anti-SRP titers in relation to age, sex, statin exposure, and CK level. The concentrations of both antibodies were not correlated with symptoms, CK level, or statin exposure.

All patients positive for anti-HMGCR antibodies were negative for anti-SRP antibodies. Eleven (12.9%) patients had anti-HMGCR antibodies. We found a tendency ($P = .051$) toward greater anti-HMGCR positivity in women (Table 1) with no symptoms. Twelve (14.1%) patients had anti-SRP antibodies. There was no sex predominance, and only 1 patient had muscle complaints.

Analyzing groups A and B separately, 57 (67%) patients had been exposed to statins (group A), whereas 28 (33%) had not been previously exposed (group B). The mean age of group A was 61.1 years (median 61 years), consisting of 20 men (median 62 years' old) and 37 women (median 60.5 years' old). Group B presented a mean age of 39.5 years (median 40.5 years), consisting of 10 men (median 31 years' old) and 18 women (median 44 years' old).

In regard to the morphological features of the muscle biopsies, 18 (54.5%) showed no specific findings, 2 had structural congenital myopathy, 4 had mitochondrial findings, 6 had idiopathic inflammatory myopathies, 1 had glycogenosis type V, 1 had lipid accumulation, and 1 had neurogenic atrophy.

Table 1

Clinical and morphological findings in patients anti-HMGCR/anti-SRP reagents.

Data	n (%)	Anti-HMGCR reagent		P*	Anti-SRP reagent		P*
		n	% (95% CI)		n	% (95% CI)	
Sex							
Men	30 (35.2)	1	3.3 (–3.4; 10.1)	.051*	6	20.0 (4.8; 35.2)	.25
Women	55 (64.7)	10	18.2 (7.6; 28.7)		6	10.9 (2.4; 19.4)	
Statin							
Unexposed	28 (32.9)	3	10.7 (–1.5; 22.9)	.668	6	21.4 (5.2; 37.6)	.175
Exposed	57 (67.1)	8	14.0 (4.7; 23.3)		6	10.5 (2.3; 18.7)	
Muscle biopsy							
No specific findings	16 (48.5)	2	6.3 (–7.1; 19.6)	.582	2	12.5 (–5.7; 30.7)	—
Specif myopathy	17 (51.5)	1†	11.8 (–5.3; 28.8)		0	0	

HMGCR = 3-hydroxy-3-methylglutaryl-coenzyme A reductase, SRP = signal recognition particle.

* Qui-quadrado.

† Immune-mediated necrotizing myopathy.

Group A (statin-exposed) presented 8 (14%) samples which were positive for anti-HMGCR antibodies and 6 (10.5%) which were positive for anti-SRP antibodies. Among the patients with anti-HMGCR antibodies, only two had undergone muscle biopsies, one of which showed no specific findings and the other showed characteristics compatible with IMNM. Among the patients with anti-SRP antibodies, none had undergone a muscle biopsy. Of those patients who were negative for anti-HMGCR antibodies, 9 (45%) showed no specific features, whereas 11 (55%) had specific myopathy. Among the patients who were negative for anti-SRP antibodies, 9 (40.9%) showed no specific findings, whereas 13 (59.1%) showed a specific myopathy (Table 1).

Group B (statin-unexposed) presented 3 (10.7%) patients who were positive for anti-HMGCR antibodies and 6 (21.4%) who were positive for anti-SRP antibodies. Among the patients with anti-HMGCR antibodies, 1 had undergone a muscle biopsy revealing no specific findings. Of the patients with anti-SRP antibodies, 2 had been submitted to muscle biopsy, also showing no specific findings. Among patients who were negative for anti-HMGCR antibodies, 10 (40%) had undergone muscle biopsy, with 4 (40%) patients showing specific myopathy, whereas 6 (60%) had no specific findings. In contrast, among patients negative for anti-SRP, 5 (55.6%) had no specific findings and 4 (44.4%) showed specific myopathy (Table 1).

Muscular symptoms were present in 31 (36.5%) patients, 4 (12.9%) were positive for anti-HMGCR antibodies and 1 (3.2%) was positive for anti-SRP antibodies. On the contrary, 54 (63.5%) patients had no muscle symptoms, 7 (13%) were anti-HMGCR positive, and 11 (20.4%) were anti-SRP positive. We found statistical significance for patients with anti-SRP antibodies when asymptomatic and symptomatic patients were compared ($P = .029$). In contrast, there was no statistically significant difference between symptoms and positivity for anti-HMG antibodies (Fig. 1).

4. Discussion

The aim of this study was to analyze different aspects of a heterogeneous cohort of 85 patients in relation to the presence of anti-HMGCR and anti-SRP antibodies. Patients were split into 3 groups: those with muscle symptoms and/or elevated CK, statin-exposed, and statin-unexposed; current statin users without neuromuscular symptoms; and healthy volunteers. The cohort was classified into 2 groups based on whether they had been exposed to statins (group A) or not (group B).

In terms of subject age, the statin-exposed group were older than the statin-unexposed group. In addition, the majority of patients positive for anti-HMGCR antibodies (n = 8) belonged to this group.

Previous studies have used a more specific series of patients, such as those with myositis, particularly IMNM, to test the specificity of anti-HMGCR and anti-SRP antibodies for that particular disorder. These samples were compared with healthy control volunteers, either patients with other connective tissue diseases or statin users.[9–11]

Our results did not show any significant correlations among the different parameters (Table 1). We found that 14% of patients positive for anti-HMGCR antibodies were in group A (statin-exposed) and 10.7% were in group B, and there was no significant difference between the groups. This indicates that statin exposure does not induce the production of anti-HMGCR antibodies ($P = .668$). The same was observed in relation to

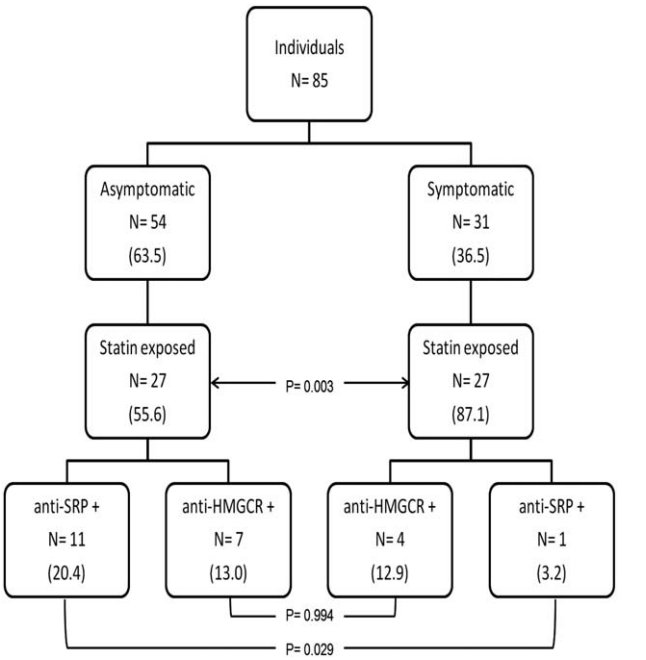

Figure 1. Statin-exposed x anti-HMGCR x biopsy findings. HMGCR = 3-hydroxy-3-methylglutaryl-coenzyme A reductase, SRP = signal recognition particle.

anti-SRP antibodies. In group A, we found that 10.5% of patients had anti-SRP antibodies, whereas 21.4% of patients in group B had anti-SRP antibodies ($P = .175$).

Anti-SRP autoantibodies have been estimated to occur in around 4% of patients with autoimmune myopathy; however, these antibodies might not be entirely specific for patients with this myopathy. At least 2 studies have reported that patients with systemic sclerosis or rheumatoid arthritis had anti-SRP antibodies but not muscle weakness.[9]

In our sample, 14.1% of individuals had anti-SRP antibodies. Muscle symptoms were present in 36.5% (n = 31) of statin-exposed patients in our cohort. Just 1 patient was anti-SRP reagent.

It is known that anti-HMGCR antibodies are specific to IMNM, with or without previous statin-exposure. In 2012, the prevalence of anti-HMGCR antibodies in 1966 participants (including 763 current statin users) was determined in a community-based study of atherosclerosis risk including 98 French Canadian subjects with familial hypercholesterolemia, of whom 51 had documented statin intolerance. The majority of patients with and without statin exposure did not develop anti-HMGCR antibodies.[10]

In 2014, Musset et al studied a total of 20 samples from myositis patients who were previously positive for anti-HMGCR antibodies and 20 negative controls using various methods. Nine of the 20 anti-HMGCR-positive patients were taking statins.[11]

In 2015, a Chinese study evaluated the presence of anti-HMGCR antibodies in 405 patients with inflammatory myopathies (IMs) including 90 healthy controls and 221 patients with other rheumatic diseases. Of the 405 IM patients, 22 (5.4%) were found to carry anti-HMGCR antibodies. These patients were predominantly female (73%), and only three anti-HMGCR antibody-positive patients with IM had been exposed to statins.[12]

In our study, muscle symptoms were present in 36.5% (n = 31) of all patients. Most of these patients (87.1%) had been exposed to statins. However, only 12.9% (n = 4) were positive for anti-HMGCR antibodies. Two had undergone a muscle biopsy, 1 of whom showed no specific findings and the other had IMNM. Eleven cases had anti-HMGCR antibodies, 10 of which were women, and only 1 showed inflammatory findings in the muscle biopsy.

We found interesting results in relation to the presence of symptoms. The number of statin-exposed patients with muscular symptoms was significantly higher than patients with no muscular symptoms ($P = .03$); however, there was no significant difference in relation to anti-HMGCR antibodies ($P = .994$) suggesting that HMGCR antibodies are not related to the symptoms. We also found that anti-SRP antibodies were significantly higher in patients without muscular symptoms ($P = .029$). Despite this, only 1 case was positive for anti-SRP in the symptomatic group (Fig. 1).

One of the main aims of this study was to define a cutoff point in a heterogeneous population with different diagnoses. We also demonstrated that anti-HMGCR and anti-SRP antibodies are not 100% specific to IMNM. We found that 11 (12.9%) patients in our heterogeneous cohort had anti-HMGCR antibodies and 12 (14.1%) had anti-SRP antibodies.

A high specificity test is good for diagnostic purposes. Our cutoff values were applied for screening in this very heterogeneous population. When we selected cutoff values, we chose those with the highest specificity associated with a LR+ and LR– close to 1. We believe that these antibodies must be tested and be interpreted within the specific context.

This study presents some limitations. This retrospective study included a small number of patients who had not been exposed to statins (n = 28). Therefore, multiple regressions did not allow us to include multiple predictors of outcome. Despite these limitations, we have reported the prevalence of anti-SRP and anti-HMGCR antibodies in a different historical cohort of patients with neuromuscular symptoms who were or were not using statins. We recognize that prospective studies are needed to verify these findings.

Author contributions

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Writing – review & editing: Alzira Alves Siqueira Carvalho, David Feder, Fernando Luiz Affonso Fonseca.

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