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Screening for autoantibodies in chronic hepatitis C patients has no effect on treatment initiation or outcome

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SUMMARY. Autoantibodies in hepatitis C virus–infected patients may indicate autoimmune hepatitis or other immune-mediated diseases. This may impact safety and efficacy of interferon-based therapy of chronic hepatitis C. We investigated the association between a positive test result for a variety of autoantibodies and the initiation and efficacy of therapy for chronic hepatitis C. We analysed an observational cohort of 24 306 patients for an association between autoantibodies and treatment outcome. 8241 patients were tested simultaneously for antinuclear antibodies (ANA), liver kidney microsomal antibodies (LKM), smooth muscle antibodies (SMA) and antimitochondrial antibodies (AMA). Matched-pair analysis was performed matching one autoantibody-positive patient to three controls. Control patients had negative tests for all four antibodies. Analyses were performed for patients with a single positive autoantibody test and for patients with multiple positive autoantibody tests. A positive test result for ANA, LKM, SMA or AMA did not affect the physician's decision to initiate therapy with pegylated interferon and ribavirin. In addition, a positive test for one or multiple autoantibodies did not adversely affect sustained virologic response. There was no difference in fibrosis stage or alanine transaminase at baseline or during therapy irrespective of antibody status. Thyroid dysfunction was more frequent in patients with positive LKM antibodies ($P = 0.004$). Initiation of therapy for chronic hepatitis C and outcome were not affected by the presence of ANA, LKM, SMA or AMA. Routine testing of these autoantibodies seems not warranted. Determination of autoantibodies should be guided by individualized clinical decisions.

Keywords: antimitochondrial antibodies, antinuclear antibodies, autoantibodies, hepatitis C, liver kidney microsomal antibodies, smooth muscle antibodies.

INTRODUCTION

Screening for autoantibodies against a variety of cellular substrates is a widely used clinical practice in patients with hepatitis C to exclude autoimmune hepatitis or other immune-mediated diseases. Despite widespread use, recommendations to screen for autoantibodies are usually based on expert opinion or small studies reporting an increased incidence of adverse events associated with the presence of the respective autoantibody [1, 2].

Most studies report no effect of a range of autoantibodies on the outcome of interferon-based therapy [3–13]. However, there are no randomized studies available, and the size of the published cohorts is rather small with less than 500 patients included in total [1,4,5,7–9,11–13]. Only one study consisted of a larger cohort of 2675 patients [10]. Additionally, the type of interferons used (interferon alpha-2a, interferon alpha-2b, pegylated or nonpegylated) was mixed within the studies.

We have analysed a large data set of treatment-naïve patients looking first at the association between a positive test result for a variety of autoantibodies and the decision of the physician to initiate therapy. As a second step, we assessed the association between positive autoantibody

Abbreviations: ALT, alanine transaminase; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; CI, 95% confidence interval; ETR, end of treatment response; EVR, early virologic response; HCV, hepatitis C virus; IU/mL, international units/mL; LKM, liver kidney microsomal antibodies; OR, odds ratio; RNA, ribonucleic acid; SMA, smooth muscle antibodies; SVR, sustained virologic response.

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tests and efficacy of therapy for chronic hepatitis C. All treated patients received pegylated interferon alfa-2a plus ribavirin.

METHODS

The cohort described is part of a multicenter observational study, which is conducted by the Association of German Gastroenterologists in Private Practice (Berufsverband Niedergelassener Gastroenterologen Deutschlands e.V.) in cooperation with Roche, Germany, to determine the quality of treatment for chronic hepatitis C in clinical practice. Procedures were approved by the Ethical Committee of the Aerztekammer Westfalen-Lippe, and patients gave informed consent before entering the cohort. The data collection was performed online via a web-based data recording system. The quality of the data was assessed by on-site monitoring. Dosing and duration of both peginterferon alfa-2a (40KD) and ribavirin were recommended to be in accordance with the national treatment guidelines [14], but were ultimately at the discretion of the physician.

Until February 2011, online data documentation had been completed for 24 306 patients with chronic hepatitis C, of whom 12 369 (51%) received therapy with peginterferon alfa-2a (40KD) and ribavirin. At least one autoantibody test was available for 15 145 (62%) patients. All positive autoantibody results were reported qualitatively.

For the present evaluation, 8241 patients with chronic hepatitis C were selected, who were tested for the following four autoantibodies, that is, antinuclear antibodies (ANA), liver kidney microsomal antibodies (LKM), smooth muscle antibodies (SMA) and antimitochondrial antibodies (AMA). Of these, 59% of patients were men, median age was 43 years (IQR 34–51 years) and median known duration of hepatitis C virus (HCV) infection was 10 years (IQR 6–20 years). Genotype distribution was as following: 62% genotype 1, 7% genotype 2, 28% genotype 3 and 3% genotype 4. HCV RNA was <800 000 (IU/mL) in 63% of patients. The majority of patients were of Caucasian origin (76%) followed by Asian (1.6%), African (1.3%) and Hispanic (0.4%) descend. For 21% of patients, race was not available. HIV-coinfected patients were not included in the analysis.

For the matched-pair analysis, cases were defined as patients with at least one positive result for one autoantibody of the four tested autoantibodies (ANA, LKM, SMA and AMA). Control patients had negative tests for all four antibodies. Patients were matched one case to three control patients. Matched parameters were gender, age categorized as $\leq$ or > median age of 43 years, HCV genotype and baseline HCV RNA categorized to be < or $\geq$ 800 000 (IU/mL).

The effect of a positive test for a single autoantibody (ANA, LKM, SMA or AMA) was analysed *vs* controls.

As a second approach, the effect of being positive for two, three or all four autoantibodies was analysed *vs* controls.

The endpoints of the analyses were association of autoantibody status with the initiation of therapy against hepatitis C, fibrosis stage, alanine transaminase (ALT) at baseline and during therapy and the efficacy of therapy defined as sustained virologic response with HCV RNA < 50 (IU/mL) 24 weeks after the end of antiviral therapy. In addition, the incidence of thyroid dysfunction was analysed.

Alanine transaminase levels were analysed as median and as proportion of patients with ALT levels above 2× the upper limit of normal (ULN) (35 IU/L for women, 50 IU/L for men).

For statistical analysis, the Pearson chi-square test, *t*-test and multiple logistic regression analysis were used. Software used was SPSS for windows version 12.0.1 (IBN, New York, NY, USA). Software used for matching was from Dr. A Katalinic, University of Luebeck, Germany.

RESULTS

In a first step, 9161 patients without an autoantibody test were compared with 15 145 patients in whom at least one test for autoantibodies was performed. In women, a higher percentage of patients were tested for autoantibodies as compared with men. Patients with baseline HCV RNA $\geq$ 800 000 (IU/mL) were tested less often for autoantibodies. There was no significant difference in age, genotype distribution or level of ALT at baseline between patients tested or not tested for autoantibodies (Table 1).

Prevalences of autoantibodies in all patients tested were 17.6% for ANA, 1.2% for LKM, 4.9% for SMA and 1.9% for AMA.

In multiple logistic regression analysis, the presence of ANA was higher in older patients (OR 1.008, CI 1.004–1.011, $P < 0.001$), women (OR 1.284, CI 1.168–1.411, $P < 0.001$) and patients with HCV genotype 1 (OR 1.157, CI 1.043–1.283, $P < 007$). HCV RNA and ALT were not associated with the presence of ANA. All other autoantibodies did not show an association with demographic parameters.

In the next step, 8241 patients with simultaneous tests for ANA, LKM, SMA and AMA were tested in a matched-pair analysis. Overall, 23% of the patients in the cohort had a liver biopsy. Patients tested positive for ANA had more frequently a liver biopsy (30%) compared with controls (23%, $P < 0.0001$). However, there was no difference in fibrosis stage between patients tested positive for ANA or any other tested autoantibody and controls.

In a first matched-pair analysis, a single positive test result for ANA or LKM or SMA or AMA was not associated with a lower likelihood to initiate therapy for chronic hepatitis C with pegylated interferon and ribavirin (Fig. 1).

Table 1 Comparison of patients with or without autoantibody tests

	Patients with test for autoantibodies	Patients without test for autoantibodies	*P* value
Number of patients	15 145	9161	
Men	8843 (58%)	5618 (61%)	0.001
Women	6302 (42%)	3543 (39%)	
Age (years)	43 (34–52)	43 (34–52)	n.s.
Genotype 1	8972 (59%)	5327 (58%)	n.s.
Genotype 2	956 (6%)	547 (6%)	
Genotype 3	3829 (25%)	2395 (26%)	
Genotype 4	456 (3%)	283 (3%)	
Caucasian	11 532 (76%)	7137 (78%)	n.s.
African	203 (1.3%)	113 (1.2%)	
Asian	238 (1.6%)	121 (1.3%)	
Hispanics	62 (0.4%)	31 (0.3%)	
Unknown	3110 (21%)	1759 (19%)	
Baseline ALT (IU/L)	69 (44–118)	68 (43–117)	n.s.
Baseline HCV RNA $\geq$ 800 000 (IU/mL)	5268/14 097 (37%)	3136/8046 (39%)	0.018

ALT, alanine transaminase; IU/L, international units per litre; HCV RNA, hepatitis C virus ribonucleic acid; n.s., not significant with $P > 0.05$.
Data shown as numbers (%) or median (interquartile range) where applicable.

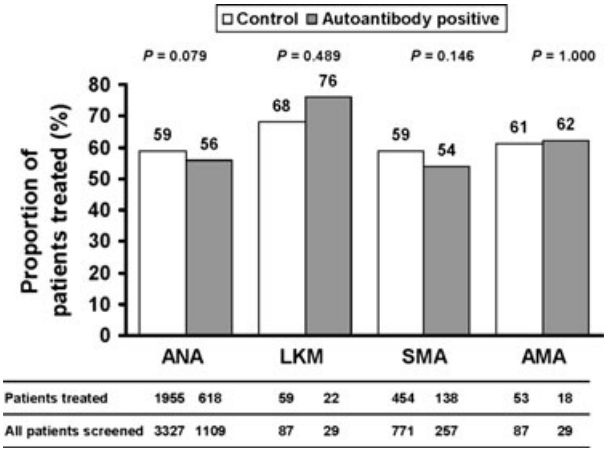

Fig. 1 The presence of a single positive autoantibody test did not affect the initiation of therapy. All patients were tested for antinuclear antibodies (ANA), liver kidney microsomal antibodies (LKM), smooth muscle antibodies (SMA) and antimitochondrial antibodies (AMA). Control patients with negative tests for all four antibodies were compared with patients positive for one autoantibody and negative for the other autoantibodies. Matched-pair analysis was performed matching one autoantibody-positive patient to three controls.

In a second approach, the association of being tested positive for ANA, LKM, SMA or AMA with response to HCV therapy was assessed (Table 2). No association with a positive autoantibody test and early virologic response (EVR), end of treatment response (ETR) and relapse rate was observed. Consequently, sustained virologic response (SVR) was observed in 51% ANA-positive patients compared with 50% of control patients ($P = 0.675$). In patients positive for antibodies against LKM, SVR was observed in 55% *vs* 55% in control patients ($P = 1.000$). In patients positive for SMA, SVR was observed in 49% *vs* 50% of control patients ($P = 0.922$). In patients positive for AMA, SVR was observed in 61% *vs* 50% of control patients ($P = 0.586$). In conclusion, the presence of one of the four autoantibodies did not affect efficacy of therapy for chronic hepatitis C.

In a second matched-pair analysis, the effect of the simultaneous presence of at least one or more autoantibodies in one patient was analysed. Being tested positive for up to four autoantibodies (ANA, LKM, SMA and AMA) had no negative impact on the decision to initiate HCV therapy compared with controls with four negative autoantibody tests (Fig. 2).

In addition, being tested positive for one or more autoantibodies had no impact on treatment outcome compared with controls (Fig. 3). The SVR rate in the matched-pair analyses was 51% for patients with one positive antibody compared with 50% in control patients ($P = 0.619$). For patients tested positive for two autoantibodies, SVR rate was 51% *vs* 48% of control patients ($P = 0.584$). For patients tested positive for three autoantibodies, SVR rate was 63% *vs* 57% in controls ($P = 0.504$). In patients tested positive for all four autoantibodies, SVR rate was 76% *vs* 46% in controls ($P < 0.05$).

Adverse events were not more frequently reported for patients with one or multiple positive autoantibody tests compared with controls. In addition, no specific adverse

Table 2 The presence of a single positive autoantibody test did not affect HCV-treatment outcome

	ANA positive	Control	LKM positive	Control	SMA positive	Control	AMA positive	Control
All patients treated	614	1842	22	66	138	414	18	54
EVR*	400/490 (82%)	1192/1446 (82%)	13/16 (81%)	50/60 (83%)	99/109 (91%)	276/329 (84%)	13/14 (93%)	34/40 (85%)
ETR	413/614 (67%)	1239/1842 (67%)	16/22 (73%)	47/66 (71%)	101/138 (73%)	277/414 (67%)	15/18 (83%)	38/54 (70%)
SVR	313/614 (51%)	920/1842 (50%)	12/22 (55%)	36/66 (55%)	68/138 (49%)	207/414 (50%)	11/18 (61%)	27/54 (50%)
Relapse	73/614 (12%)	211/1842 (11%)	3/22 (14%)	5/66 (8%)	20/138 (14%)	39/414 (9%)	1/18 (5%)	10/54 (18%)
All discontinuations	164/614 (27%)	491/1842 (27%)	5/22 (23%)	15/66 (23%)	34/138 (25%)	104/414 (25%)	2/18 (11%)	14/54 (26%)
Virologic failure	87/614 (14%)	244/1842 (13%)	3/22 (14%)	8/66 (12%)	15/138 (11%)	46/414 (11%)	0/18 (0%)	5/54 (9%)
Adverse events	37/614 (6%)	105/1842 (6%)	0/22 (0%)	3/66 (5%)	7/138 (5%)	19/414 (5%)	0/18 (0%)	2/54 (4%)
Lost before ETR	21/614 (3%)	80/1842 (4%)	1/22 (5%)	2/66 (3%)	5/138 (4%)	16/414 (4%)	1/18 (6%)	3/54 (6%)

ANA, antinuclear antibodies; LKM, liver kidney microsomal antibodies; SMA, smooth muscle antibodies; AMA, antimitochondrial antibodies; SVR, sustained virologic response; HCV RNA, hepatitis C virus ribonucleic acid.
Early virologic response (EVR) decay more than 2 log or HCV RNA < 50 (IU/mL) 12 weeks after the start of antiviral therapy; End of treatment response (ETR) HCV RNA < 50 (IU/mL) at the end of treatment; SVR = HCV RNA < 50 (IU/mL) 24 weeks after the end of antiviral therapy.
*EVR data not available for all patients.

events suggestive of autoimmune disease except thyroid dysfunction were identified.

No difference in ALT levels during therapy was observed between patients with one or multiple positive autoantibody tests and controls.

An association was observed with a positive autoantibody test for LKM and thyroid dysfunction (n = 5/29, 17%) compared with matched controls (n = 1/81, 1%) ($P < 0.01$). No association was observed for the other autoantibodies.

DISCUSSION

In this large observational cohort, testing for autoantibodies was frequent. As expected, patients being tested positive for ANA were older and more frequently women. In addition, being positive for ANA was associated with HCV genotype one infection. There was no association of autoantibodies with ALT levels arguing against a strong additional necroinflammatory effect in the liver. In addition, there was no higher proportion of patients noted with ALT flares under therapy in the autoantibody-positive subgroup.

A minority of patients (about 23%) underwent liver biopsy. Patients being positive for ANA were more frequently biopsied, but fibrosis stage was not different for patients with one or multiple positive autoantibody tests.

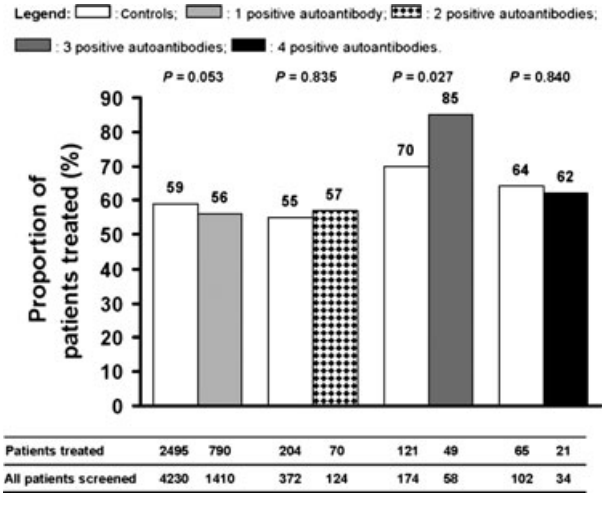

Fig. 2 No effect of one or multiple positive autoantibody tests on the initiation of hepatitis C virus (HCV) therapy. All patients were tested for antinuclear antibodies (ANA), liver kidney microsomal antibodies (LKM), smooth muscle antibodies (SMA) and antimitochondrial antibodies (AMA). Control patients with negative tests for all four antibodies were compared with patients positive for one or multiple autoantibodies. Matched-pair analysis comparing one patient with a single or multiple positive autoantibody tests to three controls showed no effect on the initiation of hepatitis C virus (HCV) therapy.

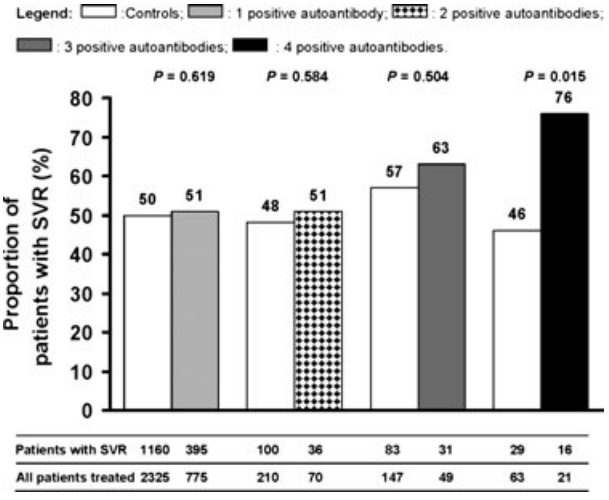

Fig. 3 Effect of one or multiple positive autoantibody tests on sustained virologic response rate. All patients were tested for antinuclear antibodies (ANA), liver kidney microsomal antibodies (LKM), smooth muscle antibodies (SMA) and antimitochondrial antibodies (AMA). Control patients with negative tests for all four antibodies were compared with patients positive for one or multiple autoantibodies. Matched-pair analysis comparing one patient with one or multiple positive autoantibody tests to three controls showed no effect on sustained virologic response (SVR) = hepatitis C virus ribonucleic acid (HCV RNA) < 50 IU/m at 24 weeks after the end of antiviral therapy.

Being tested positive for one or multiple autoantibodies did not seem to affect the decision of the physician to treat these patients. This observation raises the question why testing for autoantibodies was performed, if it did not result in a clinical decision. The impact of the result of autoantibody tests on the initiation of HCV therapy has not been assessed in studies published previously due to the different design of these cohort studies [1–12]. For this, an advantage of this cohort is the inclusion of a screening phase before the decision was taken to treat or not to treat chronic hepatitis C.

In addition, the presence of autoantibodies did not affect the outcome of interferon-based therapy for chronic hepatitis C. The lack of impact on efficacy is in agreement with most recently published reports [1–12]. However, all these cohorts were markedly smaller in size and often used a mix of interferon-based regimens including pegylated and nonpegylated interferon or interferon alfa-2a and alfa-2b without adjusting for this. The present study consists of a cohort of patients treated homogenously with pegylated interferon alfa-2a and ribavirin. In addition, most published results are based on crude cohort data. In our analysis, a matched-pair analysis reduced the bias in some important variables such as gender, age, HCV genotype and HCV RNA at baseline. In accordance with this observation apart from thyroid dysfunction, no specific autoimmune phenomenon was reported as adverse event in patients with positive autoantibody tests.

Another advantage of the present analysis is the option to assess the effect of multiple positive autoantibody tests in one patient to further explore the relevance of this phenomenon. Due to the large number of patients with autoantibody tests, a meaningful size of such patients could be evaluated. Analysing the effect of a combination of multiple positive autoantibody tests further confirmed the lack of effect on SVR rate in this cohort. This is in conflict with the observation from a much smaller cohort [1].

In this cohort, the presence of LKM autoantibodies was associated with a higher proportion of thyroid dysfunction, which is in accordance with the literature [15]. However, despite the high number of patients tested for LKM without thyroid dysfunction, that is, 8010 patients from a total of 8241, a bias due to preferential testing of patients with thyroid dysfunction for LKM autoantibodies cannot be completely excluded.

In our opinion, the main limitation of the present study is the observational nature of the cohort, which may result in a bias by selecting patients for autoantibody testing as the decision was at the discretion of the treating physician. This is, however, shared with all published papers on this topic.

However, the large number of patients included allowed a matched-pair analysis with a meaningful sample size, and there were no substantial differences in demographics, HCV genotype, HCV RNA, race or ALT level in patients with or without autoantibody testing. The analysis by race is limited as the vast majority of patients are of Caucasian origin.

Another limitation of the study is the lack of information on IL28B polymorphism due to the historic nature of the cohort and the noninterventional design.

In conclusion, based on our findings and the published literature, routine testing of autoantibodies before initiating interferon-based therapy seems not warranted, as this did not affect the decision to initiate therapy nor did it have any impact on the outcome of therapy. The determination of autoantibodies should therefore be guided by individualized clinical decisions based on the suspicion of overlap syndromes with autoimmune diseases.

AUTHORS' DECLARATION OF PERSONAL INTERESTS

S Mauss - Speakers bureau: Bristol-Myers-Squibb, Gilead, Janssen, MSD, Roche; Advisory board: Bristol-Myers-Squibb, Gilead, Janssen, ViiV. G Moog - Advisory board: Roche. D Hueppe - Speakers bureau: Bristol-Myers-Squibb, Janssen, MSD, Roche; Advisory board: Bristol-Myers-

Squibb, Gilead, Janssen. H Pfeiffer-Vornkahl - contract worker for Roche Pharma AG. U Alshuth - Employee Roche Pharma AG. F Berger, A Schober, R Heyne, C John, S Pape - None.

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