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Anti-golgi antibodies: Prevalence and disease association in Chinese population

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ABSTRACT

Objective: Anti-golgi antibodies (AGAs) are rarely encountered and often considered in relation to autoimmune diseases in clinical practice. This research was performed for studying the prevalence and clinical significance of AGAs in Chinese population.

Methods: We retrospectively reviewed 22,619 laboratory reports of AGAs detected by indirect immunofluorescence (IIF) were consecutively collected from the First People's Hospital of Wenling between June 2012 and June 2017. Eight patients with AGAs were followed up for relevant clinical and laboratory characteristics.

Result: A total of 22,619 laboratory reports were collected. Of 19 patients with AGAs, 7 cases (all females) had autoimmune diseases (AID) and 12 cases (6 females and 6 males) had non-AID. High titer AGAs ranging from 1:1000 to 1:3200 were persistently present in AID patients, while low-titer AGAs ranging from 1:100 to 1:320 were transient in non-AID patients.

Conclusion: This is the first study to assess the AGA positive rate and relevant clinical manifestations in a hospitalized Chinese population. AGAs were rare and occurred in a variety of diseases. They were persistently strongly positive in AID, whereas low-titered and transient in non-AID.

1. Introduction

Autoantibodies are particularly useful for diagnosing and monitoring autoimmune diseases (AID) and are widely regarded as characteristic serum biomarkers [1]. Certain specific autoantibodies have been suggested as serological biomarkers of some systemic AID or organ-specific AID, while some specific autoantibodies also appeared in infection diseases [2], neoplastic diseases [3] and even in healthy groups [4]. Anti-golgi autoantibodies (AGAs) detected by indirect immunofluorescence (IIF) were first described in a Sjogren's syndrome patient with lymphoma in 1982 [5]. Several studies, thereafter, had revealed that AGAs appeared as well in other AID, viral diseases [6], neurological diseases, et al. [7]. However, there had been few studies concerning the prevalence and clinical significance of AGAs in Chinese population. We therefore performed a retrospective analysis of 22,619 laboratory reports, which were requested for routine anti-nuclear antibodies (ANA) testing in the First People's Hospital of Wenling between June 2012 and June 2017, including outpatients and inpatients. Some of the patients with AGAs were followed up and analyzed in detail to deepen the understanding of the clinical and laboratory characteristics of AGAs.

2. Patients and methods

2.1. Patients

The ethics were approved by the board of the First People's Hospital of Wenling (Zhejiang, China) and informed consents were obtained from all participants, who had been followed up. A total of 22,619 consecutive data, including outpatients and inpatients, were collected from laboratory reports for detection of ANA in the First People's Hospital of Wenling between June 2012 and June 2017. In these patients, there were 5561 males and 17,058 females with a mean age of 43 ± 15 (range 1–88 years old) and 41 ± 17 (range 1–91 years old) respectively. In the reports, 7176 patients were from Rheumatology Department, 4319 patients from Neurology Department, 2450 patients from Infection Department, 1814 patients from Dermatology department and 6860 patients from the other departments. In respect to patients with AGAs, their detail clinical data were also collected, including sex, age, laboratory indexes, clinical diagnosis, clinical manifestation, such as joint pain, arthritis, liver function. The diagnosis of AGAs positive patients were all corresponded with diagnostic criteria. Diagnoses of rheumatoid arthritis (RA) was based on the classification criteria of the American College of Rheumatology (ACR)/

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European League Against Rheumatism (EULAR) classification criteria [8]. Systemic lupus erythematosus (SLE) was diagnosed according to the ACR criteria [9], Sjogren's syndrome (SS) was diagnosed according to the classification criteria of the American-European Consensus group [10], and Polymyositis (PM) was defined according to the criteria described as Bohan et al. [11,12].

2.2. Determination of AGA

AGAs, as well as ANA, were detected by means of IIF, which used HEp-2 cells and primate liver as substrate, as described by Fritzler et al. [13]. The diluted serums were firstly incubated with the HEp-2 cells grown on coverslips and primate liver, and the bound antibodies were then incubated with FITC labelled Goat-anti-Human IgG. Finally, the characteristics of fluorescence patterns were observed through a fluorescence microscope. All the detection followed the request of operation manual and a titer of 1:100 was taken as a positive reaction, and the results were confirmed by experienced technicians. When the 1:100 dilution was strongly positive and the 1:1000 dilution negative, the titer had been considered as 1:320.

2.3. Determination of ANA specific antibodies

ANA specific antibodies were detected by mean of immunoblotting (EURO line Immunoassay). ANA specific antibodies include 15 specific antibodies as follows: anti-nRNP/Sm, anti-Sm, anti-SSA, anti-Ro52, anti-SSB, anti-Scl70, anti-PM-Scl, anti-centromere protein B, anti-Jo-1, anti-proliferating cell nuclear antigen, anti-dsDNA, anti-nukleosome, anti-Histone, anti-rRNP and anti-Mitochondrial M2 antibodies.

3. Results

3.1. The fluorescence patterns of anti-golgi antibodies

AGAs showed a cytoplasmic pattern with intensely colored spots surrounded the nuclear membrane, usually in polarized pattern in the interphase of HEp-2, while karyoplasm and nucleolus were negative as demonstrated as Renier et al. [14]. During cell division, the karyoplasm showed an intensely colored diffuse fluorescence, especially around the chromosome, because of dissolved golgi. The frozen sections of the primate liver tissue showed scattered granular fluorescent staining (Shown in Fig. 1).

3.2. The prevalence of AGAs

A total of 22,619 patients were examined for ANA, of which 5836 patients were ANA positive, while only 19 patients were AGAs positive. Therefore, the positive rate of AGAs was 0.08% in 22,619 serum samples, and 0.33% in positive ANA serum samples.

3.3. The distribution, relevant clinical and laboratory characteristics of AGAs patients

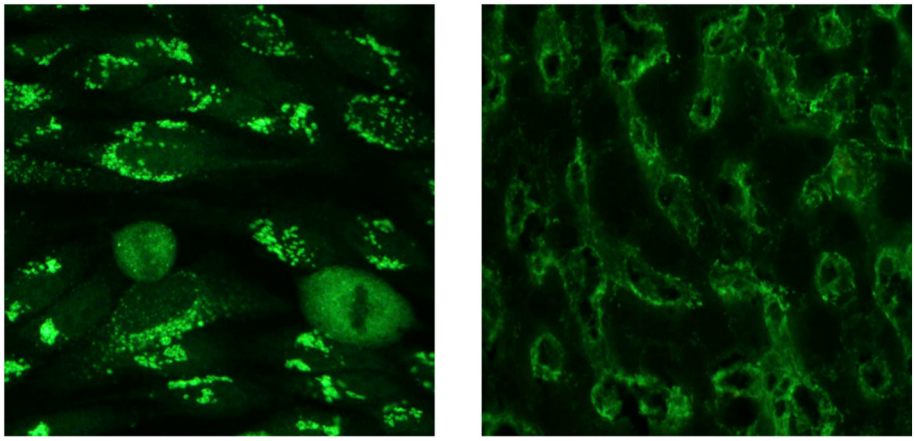
In 19 AGAs-positive patients with a mean age of 55 ± 12 (range 37 to 76), female and male patients were accounted for 13 cases and 6 cases respectively. For further analysis of distribution, the data demonstrated that AID, HBV and other diseases patients were 7, 4 and 8 cases respectively. Among the AGAs-positive patients, all patients in AID group were females, while the females and males are equal (6 and 6) in non-AID group. The AGAs titer ranged from 1:100 to 1:3200, of which 3 cases with high titers ranging from 1:1000 to 1:3200 were all observed in AID patients. The other 16 cases with low titers ranging from 1:100 to 1:320 were not only found in SS, UC and PM, but also in other diseases, such as chronic hepatitis B, tuberculosis, nodular goiter. Through analysis of relevant clinical and laboratory characteristics, we found that there were 11 patients with joint pain or arthritis, 5 cases with cardiopulmonary diseases, 5 cases with liver dysfunction, 7 cases with RF and 5 cases with ANA specific antibodies. Among the patients with ANA specific antibodies, there were 2 patients with anti-Ro52, 2 patients with anti-SSA and 1 patient with anti-histone antibody positive respectively (Shown in Table 1).

3.4. Follow-up of AGAs patients

Eight patients with AGAs were followed up for relevant clinical and laboratory characteristics, and the results were shown in Table 2. The key finding of this study was that all patients with maintained or increased AGA titers with time had non-infectious diseases such as AID, nodular goiter or cancer; whereas all patients whose AGA titers turned into negative with time had infectious diseases.

4. Discussion

Golgi apparatus is a central organelle with fascinating and complex structure in endomembrane system, and plays a key role in trafficking and sorting of protein and lipids, basic metabolism and signaling [15]. AGAs was firstly described in a Sjogren's syndrome patient with lymphoma combined in 1982 [5]. Recently, AGAs had been reported in



(a) (b)

Fig. 1. Typical fluorescence pattern of AGAs on HEp-2 cells/primate liver tissue (a: HEp-2 cells; b: primate liver tissue); original magnification ×400.

Table 1
The clinical and laboratory characteristics of patients with positive AGAs.

Case	Sex	Age (year)	AGAs titer	Clinical diagnosis	Joint pain	Cardiopulmonary diseases	Liver function	RF	ANAs
AID									
1	Female	37	1:3200	SLE	+	+	Normal	+	Anti-Ro52
2	Female	37	1:1000	SLE	+	−	Normal	−	AHA
3	Female	50	1:100	UC	+	−	Normal	−	−
4	Female	52	1:1000	RA	+	−	Normal	+	−
5	Female	58	1:320	SS	+	+	Normal	+	Anti-SSA
6	Female	69	1:320	PM	+	+	Normal	−	Anti-Ro52
7	Female	48	1:320	SS	+	−	Normal	+	Anti-SSA
No AID									
8	Female	40	1:320	Chronic hepatitis B	−	−	Dysfunction	−	−
9	Female	48	1:100	Fever, dermatitis medicamentosa	−	−	Normal	−	−
10	Female	50	1:100	Nodular goiter	−	−	Normal	−	−
11	Female	56	1:100	Ovarian cancer	−	−	Normal	−	−
12	Female	58	1:320	HBV-related HCC	+	−	Dysfunction	+	−
13	Female	62	1:100	Uterine fibroids	−	−	Normal	−	−
14	Male	45	1:100	Hepatitis B cirrhosis	−	−	Dysfunction	+	−
15	Male	58	1:100	Hepatitis B cirrhosis	+	−	Dysfunction	−	−
16	Male	63	1:320	Tuberculosis	−	+	Dysfunction	+	−
17	Male	69	1:100	Viral encephalitis	−	−	Normal	−	−
18	Male	72	1:320	Uremia	+	+	Normal	−	−
19	Male	76	1:100	Fever, renal dysfunction	+	−	Normal	−	−

Table 2
Changes in AGA titers in AID and No AID patients during clinical follow-up.

Case	Sex	Age	Clinical diagnosis	AGAs titer by first test	AGAs titer by follow-up	Follow-up time (months)
AID						
2	Female	37	SLE	1:1000	1:1000	12
4	Female	52	RA	1:1000	1:1000	18
6	Female	69	PM	1:320	1:320	11
No AID						
9	Female	48	Fever, dermatitis medicamentosa	1:100	Negative	3
10	Female	50	Nodular goiter	1:100	1:320	5
11	Female	56	Ovarian cancer	1:100	1:100	5
14	Male	45	Hepatitis B cirrhosis	1:100	Negative	10
17	Male	69	Viral encephalitis	1:100	Negative	2

various systemic AID, viral infection diseases, cerebellar ataxia, phlebitis, uremia, alcoholic hepatopathy et al. [7,16,17]. In order to improve understanding of the clinical practice of AGAs in China region, we conducted this retrospective review of the prevalence and clinical significances of AGAs.

In this retrospective analysis, HEp-2 cell and primate liver were used as antigen substrate, ANA was examined with IIF to observe the immunofluorescent patterns, showing not only nucleolar pattern, nuclear homogenous pattern, nuclear coarse speckled pattern, but also a cytoplasmic pattern with intensely colored spots surrounding the nuclear membrane, a typical fluorescence pattern of AGAs [14].Among 22,619 cases investigated, AGAs-positive rate was 0.08%, in line with the result reported by Bizzaro et al. [16]. There were some differences in positive rate with other reports [7,17,18], which might be caused by the difference of the composition of the hospitalized patients and the criterion of positive judgment of AGAs. This study determined the positive AGAs at the titer of 1:100, while the previous study at the titer of 1:80 [19]. AGAs were found to exist in multiple diseases, in addition to the common AID, such as SLE, RA, SS, PM and UC in this study. They were as well detected in non-AID, such as chronic HBV-related diseases, viral encephalitis, uterine fibroids, tuberculosis, indicating that AGAs could be detectable in a variety of diseases, as reported by other studies [5,17,18]. Among the AGAs-positive patients, all patients in AID group were females, while the females and males are equal (6 and 6) in non-AID group. This sex difference could be due to that AID was commonly seen in females.

Through analysis of the patients with AGAs, we observed that 16 patients with titers lower than 1:320 were predominantly observed in non-AID patients. The titers between 1:1000 and 1:3200 were primarily observed in patients with SLE or RA, suggesting that AGAs were not the specific characteristics of AID, especially at the low titer of AGAs. This is in accordance with the reports by Vermeersch et al. that high-titer AGAs presented persistently in AID patients [18]. Bizzaro et al. [16] hold a viewpoint that the presence of high-titer AGAs may be a signal of systemic AID, even without clinical manifestation. Long-term follow-up and studying are needed to draw a concrete conclusion.

Through the analysis of clinical manifestation and laboratory characteristic of AGAs positive patients, we found that they had joint pain, arthritis, cardiopulmonary disease, liver dysfunction, RF positive or ANA positive. However, there was no definite link between AGAs positive and the clinical manifestation observed above, and this may be related to the development of diseases. The main clinical manifestation of AGAs-positive patients with liver dysfunction were chronic hepatitis B, liver cirrhosis of hepatitis B, HBV-caused liver cancer, which were similar to the results reported by Hong et al. [17]. RF was initially reported to be related with RA but could be observed in other AID, infectious diseases and healthy population as well [8]. RF positive rate in patients with AGAs was about 50%, which was lower than the result by pooled analysis [20]. ANA in the serum sample of AGAs positive were mainly existed in the serum taken from the patients with systemic AID, and ANA specific antibodies were anti-Ro52, anti-SSA and anti-histone antibodies. The AGAs had also been reported to coexist with anti-Ro/SSA antibodies in other diseases, such as SLE, nonspecific interstitial pneumonia, myopathy, RA, etc. [20–25]. Most of the patients responded well to steroid therapy and it indicated that AGAs and ANA together influence the development and treatment response of AID. This phenomenon could contribute to the ideas concerning the treatment of patients with AGAs and anti-Ro/SSA, though the detail mechanism was worth more studies.

We had a clinical follow-up of 11 to 18 months of 3 AID patients and found that their AGAs titer presented persistently strong positive. At the 18 months follow-up, the AGAs titer of the patient with RA persisted at 1:1000 and this is consistent with the high Disease Activity Score of this patient. Thus, it is required for further study that whether AGAs had participated in the pathology and high DAS of RA. After the follow-up of 5 non-AID patients, we found that none of the patients developed

into AID, and the ANA became negative after 2 to 10 months in three of them. Among the patients, whose AGAs had turned to negative, those with fever, dermatitis medicamentosa or viral encephalitis were followed up after they had recovered. When a patient with liver cirrhosis of hepatitis B was first diagnosed with ANA positive, he was under the treatment of pegylated interferon α, resulting in a decreased copy number of HBV-DNA. AGAs of this patient also became negative three months after stopping the treatment. Therefore, this phenomenon of short appearance of AGAs in non-AID patients may be related to the antigen exposed after infection with the virus or using relevant drugs [7]. Nevertheless, the definite mechanism need to be further investigated.

There are some shortcomings to this study. Firstly, the number of the samples is limited and the data were collected from the same hospital. Secondly, the 1:320 AGA titer was not directly determined, but it was estimated only from two neighboring measurements. Moreover, this study had not elucidated the mechanism of the characteristics of AGAs positive. Finally, this study is a retrospective analysis and the influence of treatment on AGAs had not been explored. Despite the above shortcomings, this is the first study to assess the AGA positive rate and relevant clinical manifestations in hospitalized Chinese population. We demonstrated that AGAs positive rate of hospitalized patients in Chinese populations was low in clinical practice, and AGAs existed in a variety of diseases and could not act as the characteristics biomarkers for AID. However, AGAs are persistently presented strong positive in AID, but at low-titer and transient in non-autoimmune diseases, suggesting the potential role of AGAs in AID.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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