Comparison of pathological findings between patients with relapsed and non-relapsed immunoglobulin G4-related disease

Hui Lu¹, Shan Wang², Mu Wang³, Xiao-Wei Liu⁴, Lin-Yi Peng¹, Jia-Xin Zhou¹, Jie-Qiong Li³, Zheng Liu¹, Xuan Luo¹, Yu Peng¹, Yun-Yun Fei¹, Yan Zhao¹, Xiao-Feng Zeng¹, Rui-E Feng², Wen Zhang¹

¹Department of Rheumatology, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, National Clinical Research Center for Dermatologic and Immunologic Diseases, State Key Laboratory of Complex Severe and Rare Diseases, Beijing 100730, China; ²Department of Pathology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China; ³Department of Stomatology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China; ⁴Department of Ophthalmology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China.

Immunoglobulin (Ig) G4-related disease (IgG4-RD) is an immune-mediated fibroinflammatory condition characterized by elevated serum IgG4 levels and multi-organ involvement. The characteristic histological findings of IgG4-RD are dense lymphoplasmacytic infiltration, fibrosis, and massive IgG4 positive plasma cells infiltrating the affected tissues.[1] Previous studies have demonstrated various relapse predictors corresponding to demographic, clinical characteristics, laboratory test results, and treatment agents. However, no study has revealed the risk factors of flares in pathology. This study aimed to ascertain whether the pathological characteristics are related to therapeutic outcomes.

Fifteen patients, who fulfilled the 2019 classification criteria[2] of the American College of Rheumatology-European League Against Rheumatism for IgG4-RD, underwent biopsies at Peking Union Medical College Hospital, and experienced at least one relapse during the 3-year follow up, were enrolled from our IgG4-RD cohort (ClinicalTrials.gov ID: NCT01670695). Thirty non-relapsed IgG4-RD patients matched by age, sex, biopsied organ, and baseline clinical and serological parameters were selected as controls. The study was approved by the Ethics Committee of Peking Union Medical College Hospital (No. S-442). All enrolled patients consented to participate in this cohort study and provided written informed consent. All patients were treated with a standard dosage of glucocorticoids (GCs) alone or GCs combined with immunosuppressants (IMs) and followed without re-elevation in serum IgG4 concentrations.[3] Re-elevation in serological IgG4 levels alone was not considered to define a relapse.

Disease activity was evaluated using the IgG4-RD responder index (RI). Treatment response was defined as fulfilling one of the following: a decline in the IgG4-RD RI of ≥50% within 6 months or GC tapered to a maintenance dose and absence of relapse during the tapering stage.

The disease duration was defined as the interval between the appearance of symptoms and diagnosis. Organ involvement was assessed based on symptoms, signs, laboratory tests, image findings, and biopsy-confirmed diagnosis. The complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, serum IgG concentration, IgG subclasses, total IgE (T-IgE) level, and liver and renal function tests were evaluated at the time of diagnosis.

The tissues in all cases were routinely fixed in 10% neutral-buffered formalin and embedded in paraffin. Serial sections (3–4 μm thick) from each patient were prepared for hematoxylin and eosin (H&E), Elastica van Gieson, Masson’s trichrome, and immunohistochemical (IHC) staining. Two observers retrospectively reviewed all H&E sections in each case. Representative paraffin blocks were examined immunohistochemically. We identified the following typical histological features on the stained specimens: fibrosis, lymphoplasmacytic infiltration, obliterator phlebitis, and lymphoid follicle formation (ectopic germinal center).

Hui Lu and Shan Wang contributed equally to this work.

Correspondence to: Rui-E Feng, Department of Pathology, Peking Union Medical College Hospital, No. 1 Shuaifu Yuan, Dongcheng District, Beijing 100730, China E-Mail: fengruie1@163.com

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Three representative microscope fields were selected in each case. The following criteria were used to assess the degree of fibrosis: “3+” if more than half of each microscope field had been replaced by fibrous tissue; “2+” if one-third to half of each microscope field had been replaced by fibrous tissue; “1+” if less than one-third of each microscope field had been replaced by fibrous tissue; and “−” if the fibrosis was negligible. The number of the lymphoid follicles was also determined.

IHC staining was performed using the EnVision Flex IHC detection system (Dako, Glostrup, Denmark) according to the manufacturer’s instructions. The primary antibodies were a mouse monoclonal antibody against human IgG4 (EP138, 1:200; Zhongshan, China) and a rabbit polyclonal antibody against human IgG (polyclonal, 1:200; Zhongshan, China). Tonsil tissue served as a positive control. For the quantification of IgG4-positive or IgG-positive cells, the areas with the highest density of positive cells were evaluated. Five high-powered fields (HPFs) in each section were analyzed, and the average number of positive cells per HPF was calculated. One HPF covered a total area of 0.034 mm² (AXSOT microscope, 10× eyepiece and 40× lens; DPT0 camera and DPCController software; Olympus, Tokyo, Japan). The ratio of IgG4-positive plasma cells to IgG-positive plasma cells was also calculated in each case.

Continuous normally distributed data, presented as mean, was assessed using Student’s t test. Continuous non-normally distributed data, presented as median, was analyzed by non-parametric Mann-Whitney U-test after tests of normality (Shapiro-Wilk test). Categorical variables were assessed using Fisher exact test or the Chi-square test. Correlations between variables were assessed using Pearson test or Spearman rank correlation test. Statistical analysis was performed using IBM SPSS Statistics version 21 (IBM Corporation, Armonk, NY, USA). P values < 0.050 (two-tailed) were considered statistically significant. The demographic, clinical, and serological features in the two groups were shown in Supplementary Tables 1 and 2, http://links.lww.com/CMA9/A730. Age, sex, disease duration, history of allergy, distribution of biopsied organs, symptoms at disease onset, and organs involved in IgG4-RD were comparable between the groups. Regarding the serological features, the ratio of eosinophils (EO) and levels of ESR, CRP, serum IgG, IgA, IgM, IgG subclasses, T-IgE level, and IgG4-RD RI in all patients [Figure 1]. We found that the number of IgG4+ plasmacytes per HPF was negatively correlated with serum IgG2 concentration (r = −0.387, P = 0.009) and was positively correlated with serum IgG4 concentration (r = 0.32, P = 0.032), IgG4-RD RI (r = 0.492, P < 0.001), the number of involved organs (r = 0.389, P = 0.008). In addition, the number of IgG4+ plasmacytes/HPF was positively correlated with serum IgG4 concentration (r = 0.297, P = 0.048) and IgG4-RD RI (r = 0.436, P = 0.003). IgG4+/IgG+ plasmacytes ratio was negatively correlated with serum IgG2 concentration (r = −0.402, P = 0.006).

To our knowledge, this is the first study that focused on the histopathologic characteristics of patients with relapsed and non-relapsed IgG4-RD.

According to a previous study, male sex, younger age, serum IgG4 level, peripheral EO, and treatment reagents were independent risk factors of relapse. Besides, pathological characteristics vary with affected organs. Therefore, in this study, the baseline characteristics, laboratory findings, and treatment strategies were balanced between the two groups and lymph nodes were excluded because of less specific and high lymphocyte infiltration.

Common pathological variables including the number of IgG4+ plasmacytes/HPF, the number of IgG+ plasmacytes/HPF, IgG4+/IgG+ plasmacytes ratio, the degree of fibrosis, the degree of follicles, obliterative phlebitis, and eosinophilic granulocytes were comparable between the groups. We did not identify any specific markers that could help to predict a worse prognosis.

The fibrotic subtype in IgG4-RD was reported to be associated with a poor response to treatment, and in this study, the degree of fibrosis was not different in the patients with or without relapse. Therefore, we hypothesized that fibrosis might affect treatment response but not relapse.

This study had some limitations. First, the sample size was relatively small. Moreover, it was a retrospective study and the deviation might exist, although we matched the age, sex, and biopsied organs where feasible. Further studies were needed to discover pathological markers for predicting disease relapse.

**Funding**

This work was supported by grants from the National Natural Science Foundation of China (Nos. 81771757, 81870608, and the Natural Science Foundation of China (Nos. 81771757,

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Chinese Medical Journal 2021;Vol(No)
81771780, 82071839), the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (No. NWB20203346), Capital’s Funds for Health Improvement and Research (No. 2020-2-4017), and Beijing Municipal Science and Technology Commission (No. Z201100005520023).

**Conflicts of interest**

None.

**References**


