ORIGINAL INVESTIGATIONS

The diagnostic value of serum IgG4 for the diagnosis of IgG4-related disease: and is that so great?

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IgG4-related disease (IgG4-RD) is a systemic immune mediated condition that is characterized by the formation of tumor-like fibroinflammatory foci in different organs and by the elevation of serum and tissue IgG4 levels in the majority of patients. The pathogenesis of the disease, including the role of IgG4, has not been established exactly. Serum and tissue IgG4 hypersecretion is a nonspecific sign and occurs in many rheumatic, infectious, and malignant diseases.

Objective: *to determine the range of nosological entities associated with the increase in serum IgG4 levels, as well as the frequency and nature of this increase in patients with IgG4-RD.*

Patients and methods. *The results of all serum IgG4 measurements carried out in the Laboratory of Immunology and Molecular Biology of Rheumatic Diseases, V.A. Nasonova Research Institute of Rheumatology, in 2017–2018 were analyzed. Serum IgG4 parameters were separately estimated in 52 patients with verified IgG4-RD according to the universal diagnostic criteria proposed by H. Umehara et al (2011).*

Results and discussion. *In 2017–2018, a total of 247 patients were tested for serum IgG4 levels. The latter were elevated in 76 (30.8%) patients, but only 28 (36.8%) were diagnosed as having IgG4-RD. Along with IgG4-RD, anti-neutrophilic cytoplasmic antibody (ANCA)-associated vasculitis, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) were characterized by increased serum IgG4 levels. The highest median IgG4 level was found in patients with a verified diagnosis of IgG4-RD: 6.31 vs 3.2, 3.22, and 2.69 g/L in ANCA-associated vasculitis, RA, and SLE, respectively.*

In 52 patients with IgG4-RD, the IgG4 level >1.35 g/L was found in 88% of cases. The median serum IgG4 level was 3.45 g/L (2.1; 11.4). The highest level was observed in patients with generalized lymphadenopathy and in those with IgG4-related sialoadenitis and dacryoadenitis (Mikulicz disease). The serum IgG4 level was positively correlated with the number of affected organs (Spearman's correlation coefficient, 0.39; p=0.0056, Student's t-test). All the patients showed a tendency towards decreasing serum IgG4 levels during treatment regardless of its clinical response; however, the levels returned to normal only in 73% after 12 months of treatment.

Conclusion. *The increased serum and tissue IgG4 concentration is not specific, but at the moment it is the only disease marker available in clinical practice. To correctly interpret the diagnosis, it is necessary to assess the entire set of clinical manifestations, imaging data, and morpholopathological findings.*

Keywords: *IgG4-related disease; serum IgG4; Mikulicz disease; ANCA-associated vasculitis, systemic lupus erythematosus; rheumatoid arthritis.*

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IgG4-related disease (IgG4-RD) is a systemic immune mediated condition characterized by formation of tumor-like fibroinflammatory lesions in different organs, elevation of serum IgG4 levels in the majority of patients and abundant IgG4-positive (IgG4+) plasma cells in the affected tissues [1]. H. Hamano et al. were the first to discover the link between serum IgG4 hypersecretion and autoimmune pancreatitis type 1, the classical manifestation of IgG4-RD [2]. According to their results, serum IgG4 elevation >135 mg/dL had 95% sensitivity and 97% specificity in differential diagnosis of sclerosing pancreatitis and pancreatic cancer [2], but subsequent works showed much lower rates of sensitivity and specificity [3]. Further investigations demonstrated that serum IgG4 levels >135 mg/dL can also be used in diagnosis of extrapancreatic IgG4-RD [4, 5]. Currently, IgG4 level in the serum and in tissues is the main marker of this disease and is included in the comprehensive diagnostic criteria of IgG4-RD (H. Umehara et al.) [6], though its role in the pathogenesis of the disease is still unknown.

IgG type 4 is the least common (<5%) subclass of IgG in humans [7]. In physiologic conditions it can be elevated in males and elderly people; as a result of prolonged antigenic stimulation, for example, in allergic diseases, and is aimed at weakening the immune response to the antigen [7, 8]. In IgG4-RD patients, especially in those with multiple organ involvement, serum IgG4 levels can be elevated by dozens of times [9], but in a substantial number of patients (10–36%) with the active disease these levels can still be within the normal range [10–12]. Moreover, serum IgG4 elevation is nonspecific, and can be detected in many other rheumatic (rheumatoid arthritis (RA), systemic sclerosis (SSc), primary/secondary Sjogren's syndrome (SjS), systemic lupus erythematosus (SLE), Behcet's disease, ANCA-associated vasculitis), infectious and malignant diseases (leukemia, cholangiocarci-

noma, pancreatic adenocarcinoma) [10], all of which should be included in differential diagnosis.

The aim of this study was to determine the range of diseases associated with serum IgG4 elevation and to estimate the frequency and type of IgG4 elevation in IgG4-RD.

Patients and methods. We evaluated all serum IgG4 measurements carried out in the Laboratory of Immunology and Molecular Biology of Rheumatic Diseases of V.A. Nasonova Research Institute of Rheumatology in 2017–2018, and matched them with patients' clinical data. We also evaluated serum IgG4 measurements in 52 patients with IgG4-RD treated in V.A. Nasonova Research Institute of Rheumatology from 2011 to 2015. Serum IgG4 levels were measured using immune nephelometry technique (BNProSpec, Siemens, Germany) (normal range 0.1–1.3 g/L). In case of multiple tests in the same patient only the first one was taken into account. Diagnosis of IgG4-RD in all cases was proven histologically and was based on the comprehensive diagnostic criteria by H. Umehara et al. [6]: 1) organ enlargement and/or dysfunction; 2) serum IgG4 elevation >135 mg/dL (1.35 g/L); 3) lymphoplasmacytic infiltration of tissues with IgG4+/IgG+ ratio >40%. The diagnosis was definite if all 3 criteria were present; probable if serum IgG4 concentration was normal, but there was organ affection (criterion 1), and tissue IgG4-hypersecretion (criterion 3); possible if there was organ affection (criterion 1), and IgG4 was elevated only in serum (criterion 2), but not in the tissue, or if immunohistochemical analysis was not performed. In all cases the presence of at least two histological features of IgG4-RD (storiform fibrosis, lymphoplasmacytic infiltration, phlebitis, mild eosinophilia) and exclusion of other diseases (necrosing vasculitis, granulomatosis, malignancy, including lymphomas, etc.) were a must [1].

Statistical data were analyzed by means of R 3.2.2 and MS Excel (2013) programs using parametric and nonparametric analysis. To characterize the qualitative data absolute and proportionate frequencies (percent) were used; to characterize the quantitative data – mean (M) and median (Me) values with the interquartile range [25th – 75th percentile] when parameter distribution differed from normal. Two-tailed Mann–Whitney test was used for quantitative data. If there were more than two groups, Kruskal–Wallis test was used, in the case of significant differences further pair-wise comparison was made using the Nemenyi test. Fisher's exact test was used to compare the frequencies. Spearman correlation coefficient and Student's test were used to compare quantitative data. The difference was significant if $p < 0.05$.

Results. In 2017–2018 years in the Laboratory of Immunology and Molecular Biology of Rheumatic Diseases 268 serum IgG4 tests in 247 patients were performed. Serum IgG4 elevation was detected in 76 (30.8%) patients, among which 29 (38.1%) were male. In the majority of cases the test was performed due to orbital and/or major salivary glands affection, marked elevation of total serum IgG and/or gamma globulins. Diagnoses of patients with serum IgG4 elevation are listed in Table 1.

Only 28 (36.8%) out of 76 patients with elevated serum IgG4 had IgG4-RD, and in 4 patients (5.3%) it was suspected, but the biopsy of the affected organ was not performed. There was no follow-up or the information about therapy and therapeutic responses for these 4 patients either, so it was impossible to make a reasonable judgment about whether or not they had IgG4-RD. In one quarter of cases only the results of serological tests were available; these patients never underwent a rheumatologist's examination at the Institute, so there was no data about their diagnoses.

Along with IgG4-RD, serum IgG4 elevation was detected in many other rheumatic diseases, predominantly in ANCA-associated vasculitis, RA and SLE. The highest mean serum IgG4 levels were found in patients with established diagnosis of IgG4-RD (6.31 vs 3.2; 3.22 and 2.69 g/L in ANCA- associated vasculitis, RA and SLE respectively; see Table 1). In one case a malignant tumor, retroperitoneal liposarcoma, was revealed.

Among 52 IgG4-RD patients 57.7% were women. Definite diagnosis of IgG4-RD was made in 33 (63.5%) patients, probable diagnosis of IgG4-RD – in 9 (17.3%) patients, and possible diagnosis of IgG4-RD – in 10 (19.2%) patients. Multiorgan disease, i.e. involvement of two and more organs, was revealed in 39 (75%) patients. The mean number of the affected organs was 2 per patient (from 1 to 6). The most frequent sites were the lacrimal glands (33 patients, 63.5%), major salivary glands (24 patients, 46.2%), lungs (25 patients, 48%), lymph nodes (18 patients, 34.62%) and retroperitoneum (9 patients, 17.3%). Serum IgG4 elevation >135 mg/dL (1.35 g/L) was found in 88% of patients, and more than twice the upper limit – in 60% of patients. Median serum IgG4 was 3.45 g/L [2.1; 11.4]. The highest levels were observed in patients with generalized lymphadenopathy and IgG4-related dacryoadenitis and sialadenitis (Mikulicz disease).

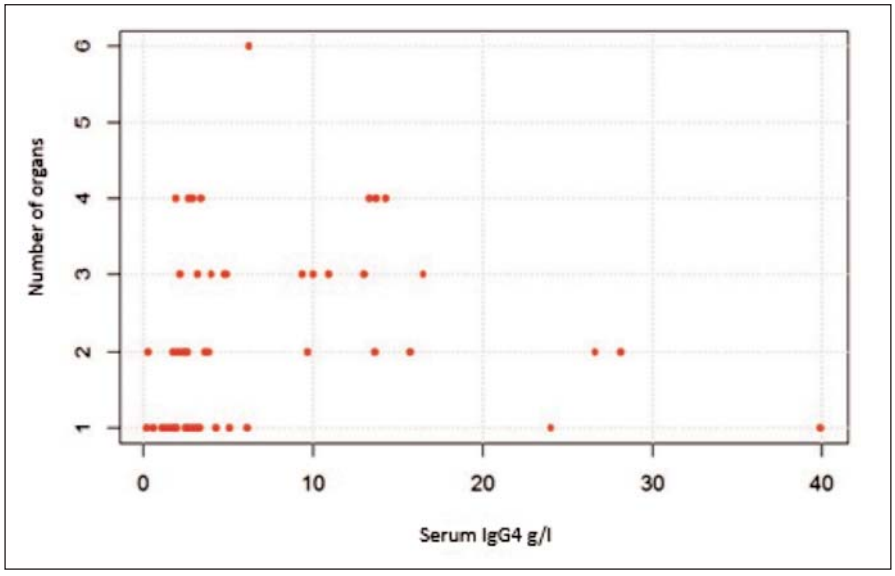

Figure 1. *Correlation of serum IgG4 level with the number of affected organs in patients with IgG4-RD.*

Serum IgG4 levels positively correlated with the number of the affected organs (Spearman correlation coefficient 0.39, p=0.0056; see Figure 1). Interestingly, in our study only the patients with isolated IgG4-related orbital disease had IgG4 levels <1.35 g/L.

Table 1. Diagnoses of patients with serum IgG4 elevation.

Diagnosis				Serum IgG4, g/L		
	Number of pts, n (%)	Male, n	Female, n	M	Me	min–max
IgG4-RD	28 (36.8)	6	22	6.31	3.76	1.44–32.7
IgG4-RD (under-diagnosed)*	4 (5.3)	1	3	3.97	3.79	1.9–6.4
No data**	19 (25)	9	10	3.49	2.1	1.36–18.3
ANCA-vasculitis	6 (7.9)	3	3	3.2	2.8	1.57–5.91
RA	4 (5.3)	3	1	3.22	3.06	2.43–4.34
Juvenile RA	3 (3.9)	1	2	2.4	2.07	1.98–3.15
SLE	3 (3.9)	1	2	2.69	2.16	1.86–4.04
Still disease	2 (2.6)	1	1	-	-	1.63–1.64
SjS	1 (1.3)	0	1	-	-	4.5
RA + SjS	1 (1.3)	1	0	-	-	2.44
Castleman disease	1 (1.3)	1	0	-	-	3.0
Idiopathic nasal septum perforation	1 (1.3)	1	0	-	-	2.1
Weber–Christian disease	1 (1.3)	0	1	-	-	2.35
Sarcoidosis+ EN	1 (1.3)	1	0	-	-	2.22
Liposarcoma	1 (1.3)	0	1	-	-	3.0
Total	76 (100)					

Note * – there was no biopsy of the affected organ; ** – clinical data, other than serology, were not available; EN – erythema nodosum.

Serum IgG4 level had no correlation with total serum IgE level (p=0.7357, Student's test for Spearman correlation) and mean serum IgG4 level had no difference in patients with fever (p=0.6146, Mann–Whitney test), patients with prior glucocorticoid treatment (p=0.7913, Mann–Whitney test) and in patients with allergic diseases (p=0.8228, Mann–Whitney test).

For convenience of comparison of serum IgG4 levels in different IgG4-RD localizations, patients were divided into 4 subgroups: 1) patients with isolated IgG4-related orbital disease (n=14); 2) patients with isolated IgG4-related sialadenitis of the major salivary glands (enlargement of at least one group of major salivary glands without evidence of orbital affection on physical and/or ultrasound examination; n=6); 3) patients with a combination of IgG4-related dacryo- and sialadenitis (on physical and/or instrumental examination; n=17); 4) patients with IgG4-related retroperitoneal fibrosis (on abdominal CT and/or MRI, n=9). Median serum IgG4 levels in these subgroups varied significantly (p=0.0086, Kruskal-Wallis test), but in a pair-wise comparison a significant difference was found only between groups 1 and 3 (p=0.009, Nemenyi test; see Figure 2). In the 1st subgroup serum IgG4 level was, on average, lower by 3.4 g/L.

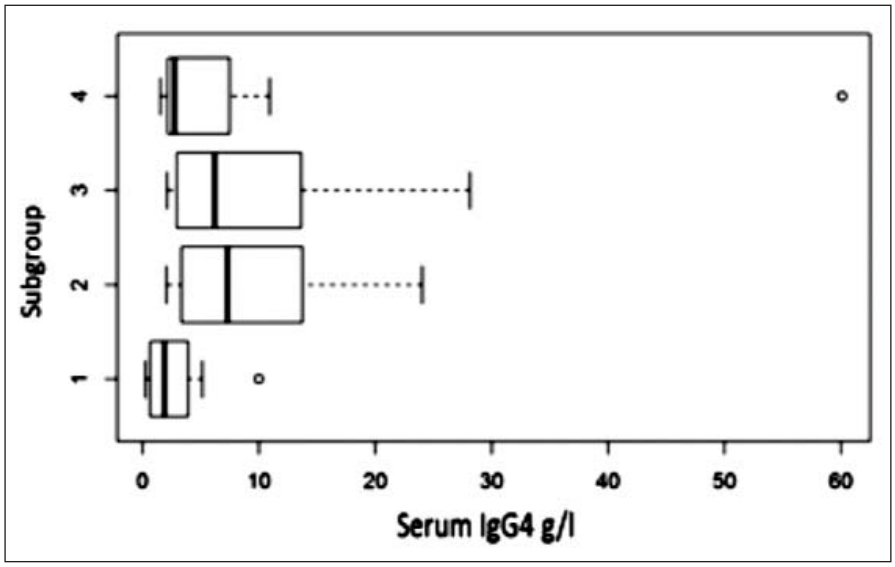

Figure 2. *Difference in serum IgG4 levels in different subgroups of IgG4-RD.*

After treatment, in all patients, irrespectively of clinical response, serum IgG4 levels declined, but even in patients with good therapeutic response IgG4 levels returned to normal within 6 months of treatment only in 57% of patients, and within 12 months of treatment – in 73% of patients.

Discussion. IgG4-related disease is a systemic fibroinflammatory immune- mediated condition with unknown pathogenesis. The role of the major disease marker, IgG of subclass 4, which is elevated in the serum of the majority of patients with active IgG4-RD, is unknown either. Development of universal diagnostic criteria is challenging due to a great variety of the affected sites and organs in IgG4-RD. In 2011 comprehensive diagnostic criteria of IgG4-RD were proposed [6], and according to these criteria, the leading role in the diagnosis belongs to detection of IgG4 hypersecretion in serum and affected tissues, but it is their weak point as well. The fact is that IgG4 hypersecretion is a nonspecific sign that can accompany many other diseases. M.N. Carruthers et al. [10] showed that in a large (n=380) non-Asian cohort of patients with serum IgG4 elevation only 34% had IgG4-RD. We have obtained similar results: in our work only 36.8% of patients with elevation of serum IgG4 above the upper limit had an established diagnosis of IgG4-RD. According to M.N. Carruthers et al. [10] detection of elevated serum IgG4 has 90% sensitivity, 60% specificity, 34% positive predictive value and 96% negative predictive value for diagnosis of IgG4-RD. If the upper limit is raised twice (up to 270 mg/dL), the specificity also raises, but sensitivity declines dramatically [13]. Moreover, in those cases when serum IgG4 is extremely high, about one quarter of test results can be false negative due to prozone effect[1] [13].

Despite a small number of patients in our study, we came across a case of malignant retroperitoneal tumor with IgG4 hyper expression not only in serum (twice the upper limit), but in the tissue as well. Overexpression of IgG4 in tissue is not a specific feature of IgG4-RD either, and it can be detected in a wide range of conditions: primary sclerosing cholangitis, ANCA-associated vasculitis, rhinosinusitis, Rosai-Dorfman disease, Castleman disease etc. [1]. J.D. Strehl et al. [14] reported that a significant number of IgG4+ cells, sufficient for immunomorphological diagnosis of IgG4-RD, can be present as a part of nonspecific chronic inflammatory infiltrate in different organs, for example, in RA synovitis, inflammatory diseases of oral cavity, some skin diseases and even can be found in peri-/intratumor infiltrate in different carcinomas.

Our data confirm that serum IgG4 levels correlate with the number of affected organs in IgG4-RD [13, 15]. That is why patients with high serum IgG4 levels need comprehensive evaluation with CT of the orbits, thorax, abdomen, retroperitoneal area and in some cases pelvic CT and/or PET/CT with 18F- fluorodeoxyglucose.

In our study 27% of patients with complete clinical and good radiologic response even after 12 months of therapy still had elevated serum IgG4 levels. This raises the question of possible use of this marker for monitoring disease activity during treatment, assessing response to therapy and detecting periods of exacerbation. Serum IgG4 measurement was excluded from the latest version of IgG4-RD responder index, because a substantial number of patients do not achieve the normal serum level of IgG4 even during the periods of remission [15].

[1]In nephelometric assay, used to measure serum IgG4, if there is overload of antigen (IgG4) the measurement can be incorrect (false negative) without sufficient dilution of the serum sample. In nephelometric assay, used to measure serum IgG4, if there is overload of antigen (IgG4) the measurement can be incorrect (false negative) without sufficient dilution of the serum sample.

Thus, there is a great demand for new IgG4-RD markers. The level of circulating plasmablasts is a promising marker, both for diagnosis and monitoring the disease activity. Plasmablasts are an intermediate stage of development between activated B-cells and plasma cells with phenotype Cd19lowCD20-CD38+CD27+ [12]. Z.S. Wallace et al. [12] reported that in patients with active untreated IgG4-RD there was a remarkable elevation of the level of circulating plasmablasts and this marker was absolutely unrelated to the level of serum IgG4. Further research is needed to find out the real role of circulating plasmablasts in the diagnosis and monitoring of IgG4-RD.

Conclusion. Serum IgG4 elevation is not specific for IgG4-RD and can be detected in many different conditions, including rheumatic and malignant diseases. Nevertheless, it is the only clinical marker of IgG4-RD available at the moment, and it is elevated in the majority of patients with the active disease. It should be taken into account in differential diagnosis, and comprehensive examination with proper visualization and serological tests should be used to exclude other conditions, primarily, cancer. The leading role in differential diagnosis belongs to pathomorphological examination. In all cases correlation of clinical and laboratory data, or clinical and radiological data is needed.

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