Review

Blood biomarkers recommended for diagnosing and monitoring IgG4-related disease. Considerations from the ERN ReCONNET and collaborating partners

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Received on October 25, 2021; accepted in revised form on January 10, 2022.

Clin Exp Rheumatol 2022; 40 (Suppl. 134): S71-S80.

Key words: biomarker, ERN, IgG4-related disease, IgG4, prediction, prognosis, fibrosis

Funding and disclaimer: page S78.

Competing interests: E.L. Culver provides consultation for Horizon Therapeutics, Sanofi and Zenus BioPharma for IgG4-RD. The other authors have declared no competing interests.

ABSTRACT

Immunoglobulin G4 (IgG4)-related disease (IgG4-RD) is a chronic, clinically heterogenous fibroinflammatory condition, characterised by an accumulation of IgG4 secreting plasma cells in affected tissues and associated with increased serum IgG4 concentrations. Despite a growing recognition of the disease among clinicians from different specialties worldwide, its indolent nature, lack of a single diagnostic test and ability to mimic other malignant, infective and inflammatory conditions, makes the diagnosis challenging. As treatment options evolve, biomarkers correlating with disease activity, predicting prognosis and response to treatment are deemed required. A multidisciplinary panel of experts from the European Reference Network for Rare and Complex Connective tissue diseases (ERN ReCONNET) and affiliated international partners have performed a narrative literature search and reviewed the current evidence of biomarkers in IgG4-RD, including immunoglobulins, cytokines, chemokines and other soluble immune mediators, and cellular components of the immune system. The aim of this paper is to provide useful information for clinicians as to the utility of biomarkers for diagnosing and monitoring IgG4-RD in clinical routine and sets out recommendations for clinical decision making.

Introduction

Immunoglobulin G4 (IgG4)-related disease (IgG4-RD) is an immune-mediated condition, characterised by fibroinflammatory lesions that occur in almost any organ, but most commonly affect salivary/lacrimal glands, orbits, pancreas and biliary tree, aorta and retroperitoneum, kidney, meninges, and thyroid gland (1). Despite growing recognition of the disease and the recent introduction of classification criteria (2), early diagnosis of IgG4-RD is still a challenge because of its multi-organ nature and the lack of specific laboratory markers (3). Although serum IgG4 elevation can provide an important hint, it is not sufficient for clinical diagnosis or appropriate disease classification. IgG4-RD can only be diagnosed with a comprehensive work-up that includes histology, imaging and serology. This often requires a multidisciplinary team to exclude potential mimics, such as malignancy, infection, or other immune-mediated conditions, *e.g.* Sjögren's syndrome or vasculitis. As treatment options evolve, monitoring the disease activity longitudinally has become central in the disease management, and typically includes a combination of findings from physical examination, laboratory investigations and diagnostic imaging (4). Furthermore, the IgG4-RD Responder Index (RI) was recently developed and validated to guide health care professionals in assessing disease activity and damage, and can be adapted to assess the efficacy of treatment in a structured manner (5).

With an improved understanding of the disease pathophysiology and availability of high-dimensional diagnostic platforms, many novel blood biomarkers have been investigated and described in IgG4-RD over the past years. However, it remains uncertain as to which labo-

ratory markers are the most helpful in the diagnostic work-up and monitoring of disease activity in clinical practice. Therefore, the overall objective of this paper is to provide current evidence about the diagnostic value and clinical relevance of available laboratory biomarkers, with special focus on diagnosis, disease activity and phenotype, prognosis and fibrosis in IgG4-RD. A panel of multidisciplinary experts performed a narrative literature search and recommend biomarkers that should be included in the management of IgG4-RD in clinical routine.

Methods

Nineteen clinicians were invited to participate in this project. The expert panel included rheumatologists, gastroenterologists and clinical immunologists, and were either members of ERN ReCONNET endorsed centres for IgG4-RD or recognised clinical experts in IgG4-RD. The methodologic approach included a review of the literature followed by a discussion among the experts on the results obtained. The narrative Pub-Med literature search was performed by using the Mesh term "IgG4-related" AND "biomarker" AND either "diagnosis", "activity", "phenotype", "prognosis", "prediction", "fibrosis" or "damage". A total of 791 papers were identified. After excluding reviews, duplicates, case reports or small case series, a title and abstract evaluation was performed, resulting in 110 articles for full text review, of which 76 articles were included in the present review. The Authors discussed the clinical relevance of all identified biomarkers and, based on the level of evidence, availability of standardised tests in clinical routine and expert opinion, suggested which of them should be recommended for use in daily clinical practice.

Results

Biomarkers for diagnosis

Laboratory biomarkers for diagnosing IgG4-RD should ideally have a high sensitivity and specificity, allowing discrimination from mimicking conditions and provide information about the clinical phenotype with potentially involved organs. Since the first report of elevated serum IgG4 concentrations in patients with sclerosing pancreatitis in 2001 (6), the majority of published articles on diagnostic biomarkers focus on serum IgG4 concentrations (7-19) (Table I). According to a large meta-analysis of nine case control studies, a serum IgG4 cut-off value ranging from 1.34 to 1.44 g/L provided a sensitivity and specificity of 87.2% and 82.6%, respectively (20). When a cut-off value of 2-fold the upper limit of normal was used (ranging from 2.70 to 2.80 g/L), the pooled sensitivity was 63% (95% CI, 60.0–66.0%), and the specificity was 94.8%. Analysis of the serum IgG4/total IgG ratio did not improve the test characteristics (21), whereas determination of the IgG4/IgG RNA ratio in peripheral blood provided superior diagnostic accuracy in IgG4-associated cholangitis/autoimmune pancreatitis (n=34) compared to serum IgG4 testing alone, both for sensitivity (94% *vs*. 86%) and specificity (99% *vs*. 73%) (22). Because polyclonal IgG4 elevation has been reported in several other conditions, such as infection, systemic vasculitis, cystic fibrosis and malignancy, the diagnosis of IgG4-RD cannot only rely on this biomarker (23). Combination analysis of serum IgG4 with other biomarkers may therefore increase the diagnostic accuracy. For example, peripheral eosinophilia, which is present in about one third of IgG4-RD patients, correlates with IgG4 levels and has a positive predictive value for IgG4-RD compared to isolated serum IgG4 increase without eosinophilia (24, 25).

There is marked B-cell activation in IgG4-RD. Other immunoglobulins are elevated, specifically IgG2 (26), IgE (27), and serum free light chains (28-30). In addition, circulating $CD19^{low}$-$CD20^{-}CD27^{+}CD38^{high}$ plasmablasts are elevated in IgG4-RD, even in patients with normal serum IgG4 levels, and described to correlate with serum IgG4 levels, the number of involved organs, and the IgG4-RD RI (31-34). However, peripheral blood plasmablast are usually not routinely assessed and may also be elevated in other inflammatory conditions, limiting the diagnostic value in IgG4-RD.

The switch toward IgG4 and IgE production is related to T-helper (Th)-2 cytokines, the source of which have been attributed to be T follicular helper 2 (Tfh2) cells specialised in the formation of germinal centres (35, 36). Tfh2 cells, but also peripheral follicular helper, Treg and subsets of cytotoxic $CD4^{+}$ T cells (CTL) are associated with IgG4-RD (37). Furthermore, cytokines or soluble mediators produced by activated innate or lymphoid cells, such as IL-1 family cytokines and receptors (38), IL-4 (35), IL-5 (39), IL-6 (40), IL-13 (40), IL-33 (41, 42), CCL18 (43), CCL26 (Eotoxin-3) (44), interferon-alpha (IFN-α) (41, 42) and B cell-activating factor of the tumour necrosis factor family (BAFF) and a proliferation-inducing ligand (APRIL) (45) are elevated in the peripheral blood of IgG4-RD patients.

Among inflammatory markers, C-reactive protein (CRP) is usually moderately elevated in IgG4-RD and tends to be associated with vascular disease. CRP is significantly increased in patients with aortic aneurysms and peri-aortitis compared to retroperitoneal fibrosis alone (11, 46). In addition, soluble IL-2 receptor (sIL-2R) concentrations are consistently increased in the majority of IgG4-RD patients (47-49), and correlate with the number of involved organs (47), serum CCL18-levels (43), but not serum IgG4 levels (48). Baseline serum sIL-2R levels were increased in all 26 patients investigated in a Japanese cohort of patients (48), and were associated with refractory and relapsed disease in a long-term Chinese study of 102 patients (49).

Autoantibodies have been identified in IgG4-RD (50). However, when tested in a clinically diverse cohort of 100 patients, frequencies of IgG4 autoantibody responses against prohibitin (10%), annexin A11 (12%), and laminin 511-E8 (7%) were not significantly different from those of disease controls (51), and therefore currently not useful for the diagnosis of IgG4-RD.

Following initial reports of hypocomplementaemia in IgG4-related tubulointerstitial nephritis (52), autoimmune pancreatitis and retroperitoneal fibrosis (53), serum complement factors have been investigated in a large Chinese cohort of 312 patients. Hypoc-

omplementaemia has been identified in 20.8% of cases and associated with more involved organs, higher IgG4-RD RI, and higher laboratory parameters, such as counts of eosinophils, inflammatory markers, IgG, IgG1, IgG3, IgG4, and IgE (54).

Notably, the 2019 ACR/EULAR classification criteria for IgG4-RD clearly mention three blood biomarkers and their relevant weight in orienting diagnosis, namely IgG4, C3 and C4, and eosinophils. In particular, serum IgG4 levels have been graded; higher levels are more suggestive of IgG4-RD and represent the major serological domain in the classification criteria, hypocomplementaemia is part of the categorical assessment with a numeric weight of six, whilst mild peripheral eosinophilia supports a diagnosis, but levels exceeding 3000/μl represents an exclusion criterion (2).

Of note, robust studies comparing levels of new biomarkers in different organ manifestations, systemic/localised IgG4-RD and in different conditions considered as mimickers of the disease are lacking to date and confirmation of preliminary results are mandatory. Thus, despite several promising new biomarkers reported in studies for the diagnosis of IgG4-RD the minimal assessment to date should include: CRP/ESR, serum IgG2, IgG4, sIgE, soluble IL-2 receptor, serum C3 and C4 levels, and eosinophil count (Table II). When available, analysis of circulating plasmablasts can be considered. Although providing important information, these biomarkers should not be used alone for diagnosis but integrated with clinical radiological and pathological domains because they lack adequate accuracy for diagnostic purposes.

Clinical phenotype and organ-specific biomarkers

Four homogeneous disease phenotypes have recently been described, after applying latent class analysis to an international cohort of patients used for developing the ACR/EULAR classification of IgG4-RD (2). These include pancreato-hepatobiliary disease (31%), retroperitoneal fibrosis with/without aortitis (24%), limited head and neck disease (24%), and Mikulicz's syndrome with systemic involvement (22%). Patients in each cluster shared distinctive clinical, epidemiological, and serological features. Distinct subsets of a proliferative (affecting the glandular and epithelial tissues) and fibrotic type have also been proposed (55). This provides physicians with a framework for improving clinical recognition and investigating biomarkers of disease.

A recent Italian observational study classified 179 IgG4-RD patients by disease phenotype to assess differences in epidemiological features and outcomes. At disease onset, pancreato-hepatobiliary disease had the highest levels of serum IgG4 and IgE concentrations, although those with Mikulicz's and systemic involvement have the highest IgG4-RI and retroperitoneal disease the lowest (56). A UK prospective cohort of 48 IgG4-pancreato-hepatobiliary patients demonstrated that elevated IgE correlated with IgG4 levels, eosinophil count, and disease relapse, and were more frequently associated with atopy (27). In an observational study of 425 Chinese IgG4-RD patients assessing disease patterns associated with peripheral blood eosinophilia, a higher eosinophil count was most associated with the presence of dacryoadenitis, sialadenitis, lymphadenopathy and skin rash (25). Those with eosinophilia were mainly male, had more active disease, more organs involved, a higher risk of relapse and a longer disease duration. Moreover, high serum IgE levels have been proposed as a potential predictor of extra-ocular muscle enlargement in IgG4-related ophthalmic disease patients (57), and significant elevations of serum IgG2 found associated with orbital IgG4-RD in comparison with non-IgG4 orbital inflammation (26).

Observational cohort studies have described the presence of hypocomplementemia in patients with IgG4-related tubulointerstitial nephritis, the most common presentation of IgG4-related renal disease (52). A large prospective study in 317 Chinese IgG4-RD, divided into those with and without hypocomplementaemia, described low C3 and C4 levels in the proliferative disease subset, specifically lacrimal, submandibular and parotid glands, paranasal sinus, pancreas and bile ducts, prostate gland and lymph nodes (54). This group were more likely to have multiple organ involvement, higher disease activity and higher eosinophils, IgG4 and IgE. In a small cross-sectional study to assess the utility of serum immunoglobulin free light chains (sFLC) in distinguishing disease phenotypes and activity in 45 IgG4-RD patients and 40 disease controls, there was no difference in sFLC between the four IgG4-RD phenotypes, or in proliferative versus fibrotic subsets, however those with renal involvement had higher kappa sFLC and lambda sFLC than other groups (28). A small study that assessed the levels of an exploratory coinhibitory checkpoint molecule T cell immunoglobulin and mucin-containing-molecule-3 (TIM-3), and its ligand, galectin-9 (Gal-9), in 59 Japanese IgG4-RD reported a higher level of TIM-3 in those with visceral organ involvement (defined as pancreas, bile duct, kidney, retroperitoneal fibrosis, aorta, lung and prostate) compared to those with head and neck disease (58). The small cohort size, single ethnicity cohort, absence of clinical follow up and lack of mechanistic assessment requires further validation. Finally, increased serum levels of IFNα and IL-33 have been reported in a Japanese cohort of 21 newly diagnosed patients with type I autoimmune pancreatitis/IgG4-RD compared to healthy controls and chronic pancreatitis patients (41), suggesting a role in the disease pathogenesis, potentially mediated by a pancreatic accumulation of plasmacytoid dendritic cells (pDCs), as previously reported (59). However, whether this feature is limited to autoimmune pancreatitis or also present in the overall population of IgG4-RD needs to be determined.

Over time, prospective performance of biomarkers in uniform cohorts of patient subtypes may be possible to establish personalised follow-up and therapeutic strategies. Based on current evidence and availability of standardised tests, it is reasonable to recommend the following biomarkers to be included in the diagnostic work-up for the evaluation of potential organ manifestations

Table I. Blood biomarkers for IgG4-RD according to type of marker.

Biomarkers	Clinical relevance	Reference	
Immunoglobulins	IgG2	Elevated in IgG4-related orbital disease compared to non-IgG4 orbital inflammation	Chan 2017 (26)
	IgG4	Elevated in 55-97% of patients, correlates with disease burden, decrease after treatment in the majority of patients, increased baseline levels predict refractory or relapsing disease	Ohara 2013 (8), Yu 2015 (9), Wallace 2016 (70), Hao 2016 (20), Kawa 2017 (12), Moon 2017 (13), Tanaka 2017 (14), Tan 2018 (15), Qi 2018 (16), Maritati 2019 (17), Aydemir 2019 (19)
	IgG4/IgG mRNA ratio	Elevated in active disease, superior diagnostic accuracy in IgG4-associated cholangitis/autoimmune pancreatitis, decreases with disease response to treatment	Doorenspleet 2016 (22)
	IgE	Increased serum levels in IgG4-RD, correlate with IgG4 levels, associated with a higher risk to relapse during follow-up, potential predictor of extraocular muscle enlargement in IgG4-related ophthalmic disease	Wallace 2016 (70), Culver 2017 (27), Tsubota 2020 (57)
	IgA	Relapsed IgG4-RD patients had significantly lower levels at baseline	Sasaki 2018 (71)
	sFLC	κ sFLC and κ/λ ratio correlated positively with the number of involved organs and IgG4-RD RI, κ sFLC and λ sFLC increased in renal involvement	Martin-Nares 2021 (28), Ikemune 2021 (29)
Cytokines	IFNα	Increased serum levels in patients with type 1 AIP, correlated with serum IgG1 and IgG4 levels, and decreased after induction of remission with GC	Minaga 2020 (41)
	IL-4	Correlates with Tfh2 and plasmablast. Decrease after GC	Akiyama 2015 (35)
	IL-5	Increased in active disease; might reflect poor prognosis.	Yamamoto 2018 (39)
	IL-6	Elevated in IgG4-related vascular disease (IgG4-related aortic aneurysms and periaortitis)	Zongfei 2020 (69)
	IL-33	Increased serum levels in patients with type 1 AIP, correlate with serum IFNα levels, decrease under GC therapy	Minaga 2020 (41)
	APRIL	Serum levels significantly increased in IgG4-RD, inversely correlate with serum IgG4 level, increase under GC therapy	Kiyama 2012 (45)
	BAFF	Serum levels significantly increased in IgG4-RD, decrease under GC-treatment	Kiyama 2012 (45)
	TNF-α	Increased baseline levels in patients with relapsing or refractory disease	Zongfei 2021 (49)
	GDF15	Increased levels in retroperitoneal fibrosis and parotid gland involvement, potential surrogate marker for tissue fibrosis	Kawashiri 2018 (80)
	ELF score	Increased in 10 patients with IgG4-RD, correlated with the number of involved organs and significantly decreased under rituximab therapy	Della-Torre 2015 (82)
Chemokines	CCL17 (TARC)	Elevated in active disease, correlates with the IgG4-RD RI	Umeda 2020 (68)
	CCL18	Significantly increased in IgG4-RD, correlates with IgG4-RD RI, serum IgG4 and sIL-2R, suggested as potential biomarker of fibrotic diseases	Akiyama 2018 (43)
	CCL26 (Eotaxin-3)	Increased levels in patients with lymphadenopathy and patients with relapsing disease	Takanashi 2021 (44)
Immune cell subsets	Plasmablasts	Elevated in active IgG4-RD, even in patients with normal serum IgG4 concentrations, positively correlate with serum IgG4 levels, the number of involved organs, and the IgG4-RD RI, 18F-FDG tissue uptake, decrease after GC treatment	Mattoo 2014 (63) Wallace 2015 (31), Berti 2017 (64), Lin 2017 (32), Lanzillotta 2019 (34)
	Eosinophils	Elevated in 29-38% of patients, associated with higher incidence of dacryoadenitis, submandibular sialadenitis and lymphadenopathy, correlate with serum IgG4 levels, hypocomplementaemia and number of involved organs, increased baseline levels in patients with relapsing disease,	Wallace 2016 (70), Culver 2017 (27) Zhang 2019 (25), Peng 2021 (54)

Biomarkers	Clinical relevance	Reference	
	$CD4^+SLAMF7^+$ T_{EM} cells	Significantly increased in IgG4-RD, decrease following GC-induced disease remission	Della-Torre 2018 (37)
	Memory B-cells	Increased levels after 6 months of GC treatment predictive for disease relapse	Lanzillotta 2018 (33)
	Tfh	Correlate with circulating plasmablast levels and Tfh infiltration in affected tissue, decrease after GC treatment	Grados 2017 (36), Kubo 2018 (65)
	Th1 and Th17	Elevated in active disease, correlated with serum IgG4 and IgG4-RD RI	Xia 2020 (66)
	Tfh1	Elevated in active disease, correlated with serum IgG4 and IgG4-RD RI	Xia 2020 (66)
	Tfh2	Elevated in active IgG4-SC and AIP, correlate with IgG4-RD RI, serum IgG4 and IgE	Grados 2017 (36), Cargill 2019 (67)
Immune receptors and complement factors	sIL-2R	Elevated in IgG4-RD, correlate with number of involved organs, associated with refractory and relapsed disease, decreases after treatment, predict patients requiring GC treatment	Akiyama 2018 (43), Handa 2018 (47), Karim 2018 (48), Zongfei 2021 (49)
	C3, C4	Low levels in approximately 20% of patients, associated with IgG4-related renal disease, lymphadenopathy and gland involvement, correlate with serum IgG4 levels, eosinophil count, the number of involved organs and IgG4-RD RI	Kihara 2013 (53), Peng 2021 (54)
	TIM-3	Increased in patients with visceral involvement, in particular biliary tract, kidney and retroperitoneum	Matsumoto 2021 (58)
	CRP	Mostly moderately increased in IgG4-RD, associated with vascular disease (IgG4-related aortic aneurysms and periaortitis), decreases after GC treatment	Yamamoto (11), Kasashima 2018 (40)

AIP: autoimmune pancreatitis; APRIL: a proliferation-inducing ligand; BAFF: B cell activating factor; CCL17: C-C motif chemokine ligand 17; CCL18: C-C motif chemokine ligand 18; CCL26: C-C motif chemokine ligand 26; CRP: C-reactive protein; ELF: enhanced liver fibrosis; ESR: erythrocyte sedimentation rate; GC: glucocorticoid; RI: responder index; GDF-15: growth differentiation factor 15; sIL-2R: soluble interleukin-2 receptor; sFLC: serum free light chains; TARC: thymus and activation-regulated chemokine.

and clinical phenotypes: serum IgG4, IgE, C3 and C4 concentrations, and eosinophils. Assessment of sFLC, IFNα and IgG2 may be considered in patients with suspected renal involvement, autoimmune pancreatitis or orbital disease, respectively (Table II).

Biomarkers correlating with disease activity

Ideal biomarkers of disease activity in chronic rheumatologic disorders should be elevated during active disease and decrease following successful induction of remission without being affected by intercurrent inflammatory or infectious conditions. With the primary goal to implement patient monitoring, several serum molecules have been proposed as biomarkers of IgG4-RD activity. Serum IgG4 level was the first biomarker of this kind and still represents the most widely used serological tool to assess disease activity in clinical practice. Serum IgG4 levels are elevated in 60-70% of active untreated patients, decline substantially with clinical improvement and typically re-increases with disease relapse (60). Yet, clinicians should be aware of the many shortcomings of using serum IgG4 alone to track disease activity. Serum IgG4 concentrations, in fact, are not elevated in up to 40% of patients with active IgG4-RD, thus becoming useless in these patients to assess disease response to immunosuppressive therapies (12, 61, 62). Moreover, IgG4 serum level remains elevated in up to 63% of cases after glucocorticoid treatment and do not rise again in flares in nearly 10% of patients (60).

To overcome these limitations and with the increasing appraisal of IgG4-RD pathogenesis, more than a dozen of novel biomarkers have been proposed, but their clinical utility has not been fully established. Circulating $CD19^{low}CD20^-CD27^+CD38^{high}$ plasmablasts – the precursors of antibody secreting plasma cells – for instance, display higher sensitivity and specificity compared to serum IgG4 because they are increased compared to healthy individuals not only in patients with elevated IgG4 but also in those with normal serum concentrations (31, 32, 34, 63, 64). Moreover, increased frequencies of circulating plasmablasts positively correlated with serum IgG4 levels, the number of involved organs and the IgG4-RD RI (32) as well as 18F-FDG tissue uptake in PET/CT scans (64), and decreased after glucocorticoid or rituximab treatment (31, 32, 34, 63), suggesting that plasmablasts might be a potentially useful biomarker for diagnosis and monitoring response to treatment (32). Still, transitory appearance of circulating plasmablasts can be observed in response to a variety of inflammatory stimuli (including vaccinations), thus being an imperfect marker of IgG4-RD activity (34). Additional potential cellular biomarkers of IgG4-RD activity with a sound pathogenic rationale include circulating Tfh cells (65), particularly Tfh1 and Tfh2 cells (66, 67) as well as Th1 and Th17 cells (66). In addition, soluble immune mediators, such as serum levels of IFNα and IL33 (41), soluble IL-2 receptor (47-49), IL-5 (39), APRIL/

Table II. Proposed blood biomarkers recommended for diagnosing and monitoring IgG4-RD according to clinical relevance.

Clinical relevance	Biomarkers recommended in clinical routine	Biomarkers to be considered
Diagnosis	IgG4, IgG2, IgE, C3/C4, sIL-2R, Eosinophils, CRP/ESR	Plasmablasts
Disease activity	IgG4, C3/C4, sIL-2R, CRP/ESR	Plasmablasts
Prognosis	IgG4, IgE, sIL-2R, Eosinophils, CRP/ESR	Plasmablasts, memory B-cells, IgA, TNF-α
Clinical phenotype and organ specific markers	IgG4, IgE, C3/C4, Eosinophils, CRP/ESR	IgG2 (orbital disease), sFLC (renal), IFNα (AIP)
Degree of fibrosis		CCL18, GDF-15, ELF

CCL18: C-C motif chemokine ligand 18; CrP: C-reactive Protein; ELF: enhanced liver fibrosis; ESR: erythrocyte sedimentation rate; GDF-15: growth differentiation factor 15; sIL-2R: soluble interleukin-2 receptor; sFLC: serum free light chains.

BAFF (45) and hypocomplementaemia (53), (54) have been reported to correlate with the number of organs involved or to mirror disease activity, being elevated in active disease compared to healthy controls and decreasing following glucocorticoid treatment. In addition, chemokines such as CCL17, CCL18 and CCL26 (Eotaxin-3) have been shown to correlate with disease burden, but their response to immunosuppressive treatment has not been fully evaluated (12, 43, 44, 68). Finally, assessment of inflammatory markers might be useful for monitoring disease activity in cases of large vessel involvement because IgG4-RD (peri)aortitis is often associated with ESR and CRP elevation but normal serum IgG4 concentrations (40, 43, 46, 69). Of note, experience with these novel biomarkers remains confined to single referral centres, and longitudinal studies in large numbers of patients are warranted to validate their utility for monitoring variations in IgG4-RD activity. Therefore, the following biomarkers may be suggested currently to monitor disease activity in clinical routine: CRP/ESR, soluble IL-2 receptor, serum IgG4, and C3 and C4 concentrations. Assessment of circulating plasmablasts may be considered (Table II).

Biomarkers related to prognosis and prediction

Prognostic biomarkers usually predict the probable course and outcome of the disease (*e.g.* disease recurrence, progression, death) independent of treatment received, whereas predictive biomarkers are measured before treatment and provide information on the probability of response to a particular therapy. The current knowledge about such biomarkers in IgG4-RD is limited and frequently controversial given the multiorgan nature of the disease and heterogeneity of therapies applied. Overall, IgG4-RD is a indolent condition characterised by a rapid response to corticosteroid therapy. However, a considerable proportion of patients experience relapses during steroid tapering and suspension requiring additional induction therapy and possibly association with conventional-synthetic disease-modifying drugs (DMARDs) for disease control improvement (1). Indeed, elevated IgG4 serum level at disease onset are associated with higher risk of relapse following glucocorticoid as well as rituximab therapy (HR 6.2, CI: 1.2, 32.0), with an increased risk especially in those patients with higher levels, who will also experience earlier relapses (70, 71). Recently, the serum IgG4 cut-off value of 8.13 g/L has been proposed to identify patients who will more probably experience relapse, with a sensitivity of 57.1% and a specificity of 95.0% in one study cohort (71). Moreover, re-elevation of serum IgG4 levels during glucocorticoid treatment was associated with disease relapse (71). Similarly, high levels of serum IgE and absolute eosinophil count at baseline have been associated with a higher risk of relapse during follow-up (70). The concomitant elevation of serum IgG4, IgE and eosinophils is uncommon, underlying the importance of measuring all of them at baseline for a proper prognostic profiling (72).

A single study (71) reported serum IgA as additional biomarker for relapse risk assessment. Patients who relapsed during follow-up exhibited significantly lower levels of IgA at baseline. Perturbations of B-cell subpopulations have also been studied as a potential predictive factor of IgG4-RD relapse and it has been observed that increase of circulating memory B cells after 6 months of glucocorticoid treatment might predict disease relapse within 2 years of follow-up, (HR, 12.24; p=0.0005), with relapse rates at 12 and 24 months of 30% and 100%, respectively (33). Recently, a proteomic study recognised CCL26 (Eotaxin-3), a potent chemo-attractant for eosinophils, as being markedly elevated in IgG4-RD with lymphadenopathy, a phenotype that was associated with a higher risk of relapse (44). Additional evidence comes from Zongfei *et al*. (49) who identified numerous potential biomarkers at baseline that predict a refractory course or relapse at follow-up in a large Chinese cohort. In particular, soluble IL-2 receptor and TNF-α were independent risk factors for refractory and relapsed disease. Despite a number of studies suggest significant baseline differences between relapsing and non-relapsing patients, suggesting possible prognostic factors, these findings were not reproduced in other cohorts (14, 73). Therefore, further studies are warranted to confirm identified prognostic biomarkers. Currently, biomarkers that may reliably help evaluating the prognosis of IgG4-RD in clinical practice include serum IgG4, IgE, soluble IL-2 receptor, and eosinophil counts. Assessment of serum IgA, TNF-α, and peripheral blood memory B-cells may be considered (Table II).

Biomarkers for fibrosis and damage

Data of blood biomarkers related to fibrosis in IgG4-RD are scarce because

they are mostly evaluated on tissue samples, and plasma levels of fibrosis biomarkers are difficult to detect (74). Moreover, there is a trend to establish correlations between mediators involved in fibrosis and activity measures, being difficult to ascertain the fibrotic process itself. Fibrotic lesions in IgG4-RD are the result of complex interactions between multiple cell types such as activated B-cell subsets, $CD4^+$ cytotoxic lymphocytes (CTL) M2 macrophages and fibroblasts which produce various pro-fibrotic cytokines (75). Among these, CCL18 has been described to induce collagen synthesis in lung fibroblasts (76, 77), and has been suggested as a potential biomarker of fibrotic diseases (78). Akiyama *et al*. previously demonstrated that serum levels of CCL18 were significantly increased in 61% of 28 patients from a clinically diverse IgG4-RD cohort and serum levels correlated with the number of affected organs, IgG4-RD RI, serum IgG4 and soluble IL-2 receptor levels, and significantly decreased after 3 months of glucocorticoid therapy (43). In addition, growth differentiation factor 15 (GDF-15), a cytokine secreted by activated macrophages, has been linked with fibrosis in the setting of different diseases like systemic sclerosis (79). In IgG4-RD, serum levels of GDF-15 were found significantly elevated in a Japanese cohort of 72 untreated patients compared to healthy controls and were associated with the presence of retroperitoneal fibrosis and with parotid gland involvement, but not with the IgG4-RD RI or the number of involved organs (80). Interestingly, serum concentrations of GDF-15 in this cohort of patients did not decrease after a median glucocorticoid treatment period of 3.5 years despite clinical responses. Another surrogate of the severity of fibrosis and degree of extracellular matrix deposition comes from the Enhanced Liver Fibrosis (ELF) score, which combines serum concentration analysis of three mediators associated with fibrogenesis, and was developed for the evaluation of chronic liver disease (81). In IgG4-RD, the ELF score was found to be increased in 10 patients with IgG4-RD compared to healthy controls, correlated with the number of organs involved, and then fell with rituximab therapy (82). These findings are consistent with the concept that B-cell depletion leads to a decrease in the degree of myofibroblast activation in IgG4-RD, along with the reduction of dimensions of the inflammatory masses on imaging studies. In conclusion, determination of CCL18, GDF-15 and ELF may be considered as potential surrogates for fibrosis in IgG4-RD in centres with expertise and appropriate diagnostic laboratories. Finally, damage can be determined by the specific function assessment of every organ affected. In this regard, specific tests that evaluate the distinct organ, like pancreatic assessment for exocrine and endocrine insufficiency, can be used to ascertain the degree of malfunction due to fibrosis.

Discussion

The recognition of IgG4-RD has expanded across medical specialties around the world over the past decade, and remarkable progress has been made in understanding the immunopathogenesis of the disease. As a result, many candidate biomarkers have been identified from large-scale investigations in addition to serum concentrations of IgG4, which still represents the most important and mostly utilised marker for diagnosing and monitoring IgG4-RD (83, 84). Novel immunologic biomarkers often lack standardised test availability, or may have low evidence levels for supporting patient decision making due to the limited number of patients investigated in IgG4-RD cohorts. In this article, a multidisciplinary panel of experts from the ERN RECONNET and affiliated international partners summarised the evidence of peripheral blood biomarkers of IgG4-RD and based on their expertise, provide recommendations for health care professionals on the utility of laboratory-measured biomarkers in clinical routine with respect to diagnosis, disease activity, prognosis, clinical phenotype and fibrosis.

Biomarkers with the highest level of evidence and wide-spread availability in clinical routine settings for managing IgG4-RD include serum levels of IgG4, IgG2, IgE, complement factors C3 and C4, sIL-2R, eosinophil count and CRP/ESR levels. These markers should not only be used to support diagnosis in the appropriate clinical context, but also, with different weightings and value, provide useful information for the potential risk of individual organ manifestations, correlate with disease activity and predict responses to treatment (Table II), and are recommended to be included in the diagnostic work-up and during follow-up.

Investigation of circulating plasmablasts may provide further value but require flow cytometry facilities and are not disease-specific. The utility of recently identified autoantibodies (anti-Galactin-3, anti-Laminin 511, Anti-Annexin A11) is currently not recommended for the diagnosis or monitoring of IgG4-RD. Established biomarkers predicting the fibrotic burden in routine clinical practice are not available yet, but determination of CCL18, GDF-15 and ELF may be considered in specialised centres with sufficient expertise. In addition, novel immunologic markers, both soluble mediators (cytokines, chemokines) and cellular components (immune cell subsets) may be considered, provided that cost and technical requirements permit its use.

While biomarker research in IgG4-RD has recently improved, disease management cannot rely on single indicators and should be complemented by established laboratory analysis and diagnostic imaging to investigate organ function and damage after careful exclusion of common neoplastic and infectious mimickers. Nevertheless, the wide-spread utility of biomarkers in IgG4-RD may contribute to optimised clinical practice and, consequently, to improved patient outcomes.

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Co-funded by the European Union

Disclaimer

This publication was funded by the European Union's Health Programme (2014-2020). ERN ReCONNET is one of the 24 European Reference Networks (ERNs) approved by the ERN Board of Member States. The ERNs are co-funded by the EC (European Commission). The content of this publication represents the views of the authors only and it is their sole responsibility; it cannot be considered to reflect the views of the EC and/or the European Health and Digital Executive Agency (HaDEA) or any other body of the European Union. The EC and HaDEA do not accept any responsibility for use that may be made of the information it contains.

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