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Laboratory diagnostics of systemic autoimmune diseases – Update 2013

Abstract: This article discusses relevant and up-to-date issues for the clinical laboratory concerning arthritis activity and monitoring of rheumatoid arthritis, details for the new disease entity of IgG4-related diseases and Sjogren's syndrome. The article puts the focus on the data of 2013.

Keywords: IgG4-related diseases; rheumatoid arthritis; Sjogren's syndrome.

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Rheumatoid arthritis

Introduction

Clinical observation and clinical examination have always been there in medicine. In addition, the art of medicine, as a concept of experience and intuition, has a high priority in the process of diagnosis and treatment of diseases. It can be assumed that this will not change in the near future. Nevertheless, ever more chemical or technical lab-based tools need to be developed to provide an objective foundation for the art of medicine.

The classification criteria for rheumatoid arthritis were established in 1987 and continue to remain in effect. Clinical observation dominates among these classification criteria, and the presence of a positive rheumatic factor merely represents one additional aspect on an equal footing with the clinical criteria (Figure 1 ACR 1987). Over time, scoring methods have been developed to objectivize disease activity, such as the Disease Activity Score for the 28 most commonly affected joints (DAS28). However, since a purely clinical examination is too coarse for many patients to provide a correct diagnosis of the different varieties of RA or to control treatment involving ever more potent and more expensive drugs, magnetic resonance imaging and power Doppler sonography (by now ubiquitous in medical offices and hospitals) have come to play an important role. These equipment-based methods are suited, in particular, to diagnose chronic rheumatoid arthritis sooner. New equipment-based methods, such as in vivo fluorescence optical imaging, have been added to the arsenal [2]. Also, the classification criteria have changed towards greater objectivity by the addition of laboratory chemical parameters (Figure 1) [3].

This development reflects the clinical and scientific observation that rheumatoid arthritis is a heterogeneous disease. Thus, we distinguish RF positive from RF negative and ACPA positive from ACPA negative variants with different progressions and prognoses. Now, there is also a third option, that of anti-carbamylated protein antibodies (anti-CarP AB). In the coming years, we will certainly gain further insights into this third autoantibody entity. The anti-CarP AB, similar to the anti-CCP AB, seem to have a predictive probability of about 30% for the clinical onset of rheumatoid arthritis in patients with arthralgia within the first year [4]. The level of the antibody titer appears to be independent of this. In addition, the autoantibodies anti-CCP and anti-CarP seem independently to determine the predictive probability of the occurrence of rheumatoid arthritis. This underlines at the present time the hypothesis of three different autoantibody systems in connection with rheumatoid arthritis.

Not only the classification criteria are given additional support by laboratory medicine; therapy control on the basis of disease activity (treat to target) is also receiving increasing support from laboratory chemistry. In the United States, a lab-chemical arthritis activity score is already in use for rheumatoid arthritis,

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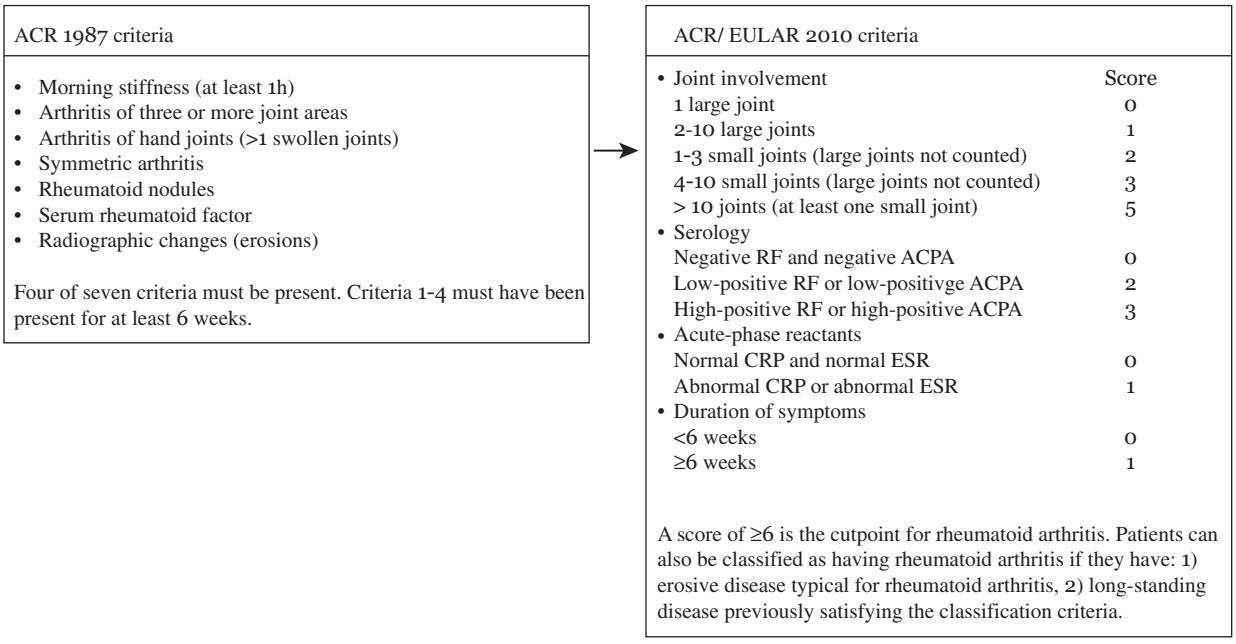

Figure 1 Markers of the non-specific, systemic inflammation CRP, ESR and relatively specific markers of rheumatoid arthritis RF and ACPA have been included in the ACR/EULAR 2010 classification criteria.
This means that in the current classification criteria, laboratory diagnostics have been given a much higher priority (modified from [1]).

the Multi-Parameter Biomarker Disease Activity Score (MBDS). Perhaps this multi-parameter test will gain importance in Germany as well.

However, for practical purposes, this also means that both the classification and diagnosis, as well as the decision of therapeutic strategy, represent a clearly clinically dominated decision in the context of rheumatoid arthritis. Lab-chemical and imaging methods cannot take the place of the clinical decision process, but support it.

Table 1 gives an overview of the development of classification/diagnostics and therapy control over the last decades. This shows clearly that equipment-based and lab-chemical diagnostics are becoming increasingly more important in routine clinical practice.

New laboratory chemical Multi-Biomarker Disease Activity Score (MBDA)

For a more objective assessment of disease activity, a 12-parameter panel of biomarkers was examined as part of the CAMERA study (Table 2), and the results were compared with the previously recognized activity parameter, the DAS28 score, via a biomarker score [5].

Before that, the MBDA score had been validated [6]. In the Dutch CAMERA study (Computer Assisted Management in Early RA), patients with early rheumatoid arthritis were evaluated in relation to the treatment response to different treatment algorithms [10]. A classic clinical parameter for arthritis activity in the course of the study was the DAS28-CRP score. This includes the number of swollen joints (SJC=swollen joint count), painful joints (TJC=tender joint count) of 28 joints examined, the CRP value (C-reactive protein) and the patients' assessment of their general health status (VAS-GH=visual analog scale of general health from 0 to 100 mm). Thus, the DAS28 score contains three relative subjective activity parameters: TJC, SJC and VAS-GH. In addition, 12 biomarkers were identified via ELISA single tests as part of the CAMERA study, which were used to obtain a more objective assessment of disease activity.

The MBDA score is based on the fact that a single marker cannot describe the different pathogenetic factors. By combining different markers that describe the corresponding biological processes in rheumatoid arthritis (Figure 2), the disease activity can be captured better in laboratory tests.

The examination times in the CAMERA study were set to baseline and after 6 months. The parameters obtained are listed in Table 2.

Similar to the DAS28 score, the MBDA score was calculated from the results of the single tests [6]. The MBDA score ranges from 1 to 100 and is classified according to the clinical significance (activity), similar to the DAS28 score, as shown in Table 3.

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Table 1 Experimental (i.e., not yet implemented in clinical routine) laboratory-chemical or equipment-based methods are not shown here.

Year	Diagnosis/classification	Therapy control
1987	ACR 1987 classification – Conventional X-ray – Clinical – RF	Clinical view
End of 1990s	ACR 1987 classification – Conventional X-ray – Clinical – RF	**DAS28 score** (TJC, SJC, ESR, VAS)
Until 2010	ACR 1987 classification – Conventional X-ray – Clinical – RF **Diagnostic help through:** → + ACPA → + Ultrasound with power Doppler → + MRI hands and feet, all three not included in classification criteria	DAS28 **Ultrasound with power Doppler** **MRI of the hands and feet**
From 2010	**ACR/EULAR 2010 early classification criteria** (and ACR 1987 criteria) – **ACPA** – **CRP and ESR** Diagnostic help through: → + Ultrasound with power Doppler → + MRI hands and feet, neither one included in classification criteria	DAS28 Ultrasound with power Doppler MRI of the hands and feet
From 2012	ACR/EULAR 2010 early classification criteria (and ACR 1987 criteria) – ACPA – CRP and ESR Diagnostic help through: → + Ultrasound with power Doppler → + MRI hands and feet, neither one included in classification criteria	DAS28 Ultrasound with power Doppler MRI of the hands and feet → **Multi-Biomarker Disease Score (VECTRA™ DA) (5, 6)**

Innovations are shown in **bold**. TJC, tender joint count; SJC, swollen joint count; VAS, visual analog scale.

Table 2 Listing the biomarkers used in the studies [5–9] and in the Vectra™ DA score (www.Vectra-DA.com).

Abbreviation	Name	Function
VCAM-1	Vascular cell adhesion molecule 1	Adhesion molecule
EGF	Epidermal growth factor	Growth factors
VEGF-A	Vascular endothelial growth factor-A	
IL6	Interleukin 6	Cytokine-related proteins
TNF-RI	Tumor necrosis factor receptor type 1	
MMP-1	Matrix metalloproteinase-1	Matrix metalloproteinases
MMP-3	Matrix metalloproteinase-3	
YKL-40	Human cartilage glycoprotein 39	Secreted by activated cells (macrophages, chondrocytes, neutrophils, synovial cells)
Leptin	Leptin	Hormones (adipocytokines)
Resistin	Resistin	
SAA	Serum Amyloid A	Acute phase proteins
CRP	C-Reactive Protein	

It was possible to find a moderate to good correlation [κ (95% CI)=0.41 (0.21–0.61)] of the DAS28-CRP response to treatment with the 12-parameter MBDA score.

In a continuation of the CAMERA study, data on the correlation of the DAS28-ESR activity score with the MBDA score were presented at the American College of Rheumatology meeting. In this context, monthly data of both scores were correlated with the baseline scores over a period of twelve months. An improvement in the DAS28-ESR correlated well with the MBDA score in both study arms, MTX-only (r=57, p<0.001, n=31) and MTX plus prednisolone (r=57, p<0.002, n=28) [7]. It has been

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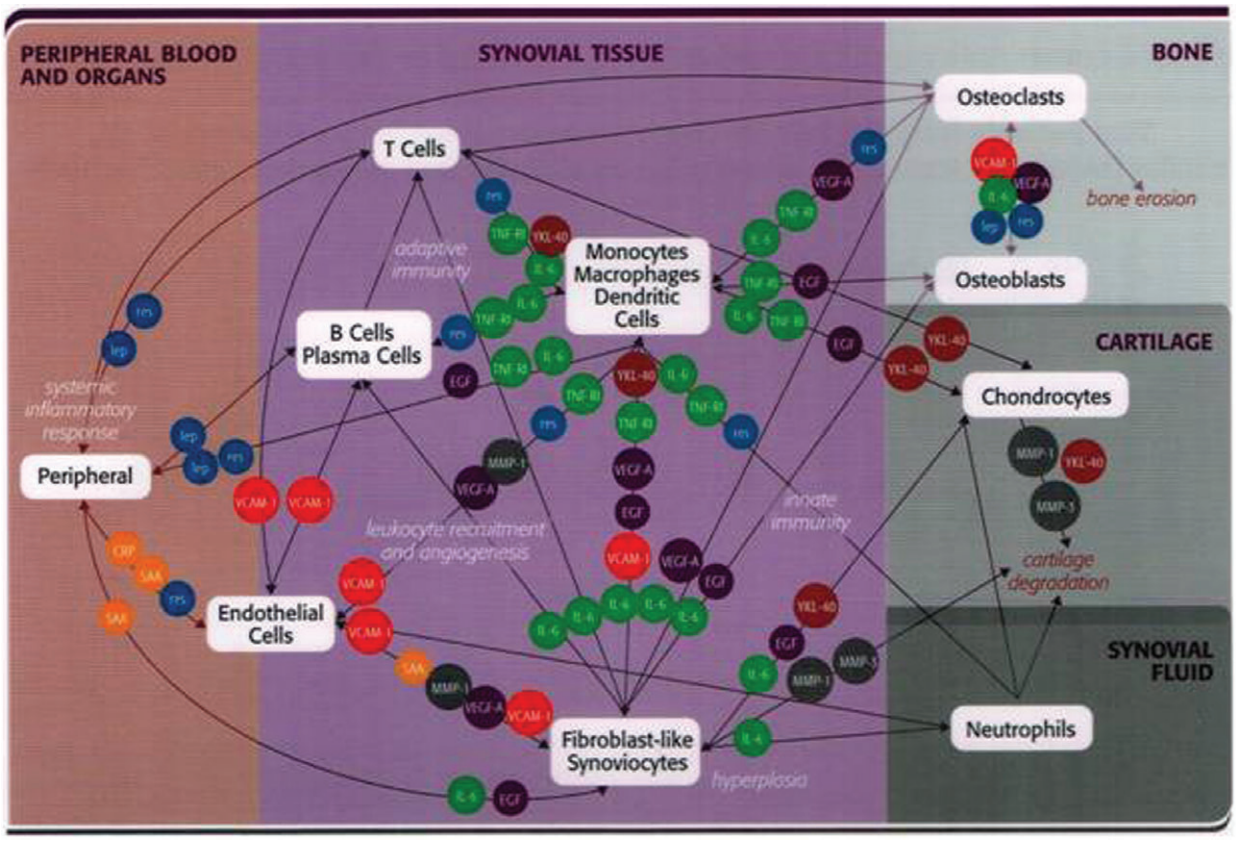

Figure 2 Biological network of rheumatoid arthritis.
This shows a biological network of rheumatoid arthritis describing the interactions of the biomarkers of the Vectra™ DA score (modified from www.vectra-da.com, Crescendo Bioscience Clinical Laboratory, 341 Oyster Point Boulevard, South San Francisco, CA 94080).

Table 3 Evaluation of the MBDA score and comparison with clinical compound score DAS28-ESR.

MBDA score	Meaning	Compared with DAS28-ESR
≤25	Remission	DAS28 remission
26–≤29	Low arthritis activity	Low DAS28
30–≤44	Moderate arthritis activity	Moderate DAS28
>45	High arthritis activity	High DAS28

shown, however, that the MBDA score is less sensitive than the DAS28-ESR score. According to Table 4, a DAS28-ESR score must improve by ≥–1.8 points in order to detect a significant improvement in the MBDA score. Changes in the DAS28-ESR score by ≥0.6 are considered to be clinically significant.

As part of this study, Eastman and colleagues tested a multiplex assay for rheumatoid arthritis with respect to its clinical applicability [8]. In previous studies, the individual biomarkers had been measured separately and compared with clinical scores [5, 6]. The preparation and validation of multiplex assay methods, however, is much more complicated in comparison to individual tests, since each antibody requires different optimal test conditions for the antigen-antibody binding. The advantage of the multiplex assay is that it takes less time and costs less money as opposed to an analysis of all biomarkers, and an analysis of one and the same blood sample. This study looked at precision, parallelism, dynamic range, cross-reactivity and the effect of interference in a 12-biomarker test for the Multiplex Disease Activity algorithm. The test analyses demonstrate very good precision without significant cross-reactivity and interference factors. Based on the properties of 12-biomarker multiplex assay, a stable multiplex test for further clinical trials is available.

The pre-analysis is crucial to the evaluation of the MBDA score [11]. Zhao et al. [11] show that antibody levels and proteins that are not released from cells, such as VCAM-1, SSA and CRP, are relatively robust in the patient's serum or plasma. However, the pre-analysis of proteins released from cells, such as EGF, VEGF, IL-6, YKL-40 and resistin, is much more susceptible to interference due to pre-analytical conditions. Pre-analysis plays a particularly prominent role in multi-biomarker tests, since errors

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Table 4 Monthly, mean change in DAS28-ESR and MBDA scores during the observation period of 12 months of the two study arms MTX and MTX + prednisolone (modified from [7]).

Timepoint	DAS28-ESR				MBDA			
	MTX		MTX+pred.		MTX		MTX+pred.	
	n	Mean Chg.	n	Mean Chg.	n	Mean Chg.	n	Mean Chg.
1 Month	16	−0.3 (p=0.24)	11	−1.9 (p<0.001)	18	−3 (p=0.27)	14	−12 (p=0.01)
2 Months	15	−0.7 (p=0.02)	11	−2.4 (p<0.001)	17	−3 (p=0.25)	14	−11 (p=0.02)
3 Months	22	−1.3 (p<0.001)	13	−3.0 (p<0.001)	25	−5 (p=0.09)	17	−15 (p=0.002)
4 Months	13	−1.8 (p<0.001)	10	−3.9 (p<0.001)	17	−9 (p=0.03)	14	−19 (p=0.003)
5 Months	15	−2.2 (p<0.001)	10	−4.2 (p<0.001)	18	−13 (p=0.006)	12	−21 (p=0.003)
6 Months	18	−2.8 (p<0.001)	12	−3.0 (p=0.001)	29	−20 (p<0.001)	19	−16 (p<0.001)
9 Months	17	−2.7 (p<0.001)	12	−3.2 (p=0.001)	24	−24 (p<0.001)	17	−20 (p<0.001)
12 Months	31	−2.8 (p<0.001)	28	−3.1 (p<0.001)	44	−20 (p<0.001)	37	−16 (p<0.001)

Solid line represents comparison with the MTX arm and dotted line represents the MTX+prednisolone arm in the comparison of DAS28-ESR with MBDA score.

in pre-analysis can lead to a shift in the clinical significance of the score result.

In practice, pre-analysis is of crucial importance for the MBDA test and the MBDA score calculation (temperature and transport time).

To what extent this test will actually have an additional benefit in the assessment of arthritis activity, is not yet fully understood. Furthermore, the question regarding its value in diagnostic monitoring for predicting a disease phase, erosivity, progression of the disease and disability has not been answered yet. Perhaps the MBDA score will evolve into a meaningful test for the progress evaluation of the activity of rheumatoid arthritis. By way of laboratory-chemical follow-up monitoring, the laboratory-chemical progression of arthritis could be checked by the family physician at shorter time intervals. If there is no reduction in the score, or if it increases considerably, the patient could see a rheumatologist sooner to have the treatment checked and adjusted. The test may well lighten the tight schedules of rheumatologists in Germany and bring about greater efficiency in terms of time and health care costs. The next few years will surely see further controlled clinical studies on rheumatoid arthritis on the basis of the MBDA score.

IgG4-related disease (IgG4-RD)

Laboratory diagnostics

The isolated determination of immunoglobulin IgG4 serum levels is not suitable to confirm or rule out an "IgG4-related disease" (IgG4-RD) [12, 13].

The new nomenclature, which combines many long-known diseases under the entity IgG4-RD, will not be discussed further here. At this point, it is sufficient to note that IgG4-RD can virtually affect any organ and is not limited to the pancreas, as is still commonly assumed.

The disease entity of IgG4-RD was first described as early as in 1961 [14], but it was not until 2001 that Hamano [15] described it as an entity in connection with autoimmune pancreatitis. Since then, the serum cut-off level of ≥135 mg/dL IgG4 has been accepted as the diagnostic level for the disease entity of IgG4-related pancreatitis.

Since the study by Hamano et al. [15], the number of publications on and the understanding of IgG4-RD have been increasing steadily, and the cut-off level of ≥135 mg/dL must be viewed from a differentiated angle today.

The publications by Ebbo et al. [12] and Ryu et al. [13] deal with the diagnostic significance of IgG4 serum levels. Across the two studies, 217 patients' data were evaluated retrospectively.

Both studies have identified numerous non-IgG4-RDs, which were also associated with elevated IgG4 serum levels (see Table 5A and 5B).

In practice, the highest mean serum levels are measured in IgG4-RDs, but given the substantial variance, it is impossible to draw definitive diagnostic conclusions from the serum level alone (see Table 5A and 5B).

Particularly chronic B-cell stimulation, such as in connection with chronic infections of the lungs and sinuses, autoimmune diseases, neoplasias and other inflammatory diseases, are measured in significantly elevated IgG4 serum levels above the previously recognized cut-off serum level of ≥135 mg/dL.

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Table 5A Spectrum of diagnoses associated with IgG4 serum levels.

Diagnosis	No. of patients (%)
IgG4-related disease	29 (18.4)
Definite	10
Probable	0
Possible	19
Respiratory diseases	32 (20.3)
Bronchiectasis	11
Chronic rhinosinusitis	7
Asthma	4
Idiopathic pulmonary fibrosis	3
Sarcoidosis	2
Other respiratory diseases[a]	5
Biliary tract diseases	26 (16.5)
Primary sclerosing cholangitis	17
Cholangiocarcinoma	6
Biliary stricture or stone	3
Pancreatic diseases	19 (12.0)
Pancreatitis, not IgG4 related	10
Pancreatic cancer	6
Other pancreatic diseases[b]	3
Cirrhosis and other liver diseases	9 (5.7)
Vasculitis	9 (5.7)
Granulomatosis with polyangiitis (Wegener's)	5
The Churg-Strauss syndrome	3
Polyarteritis nodosa	1
Atopic dermatitis	1 (0.6)
Miscellaneous diseases[c]	4 (2.5)
No specific diagnosis	29 (18.4)

[a]Other respiratory diseases (5 patients) included chronic pleuritis, emphysema, fibrosing mediastinitis, hypersensitivity pneumonitis, and recurrent pneumonias, respectively. [b]Other pancreatic diseases (3 patients) included intraductal papillary mucinous neoplasm, pancreatic cyst, and pancreatic insufficiency, respectively. [c]Miscellaneous diseases (4 patients) included lactose intolerance, neurofibromatosis, polymyositis, and psoriasis, respectively.

Table 5B Final diagnosis in patients with elevated serum IgG4 level (>1.35 g/L).

	n (%)	Mean IgG4 levels, g/L (extremes)
Repeated infections	15 (25.4%)	2.31 (1.37–4.3)
Autoimmune diseases	8 (13.6%)	3.62 (1.38–11.3)
No final diagnosis	8 (13.6%)	1.94(1.37–2.97)
IgG4-RD	**6 (10.1%)**	**12.64 (2.48–20.6)**
Possible IgG4-RD	5 (8.5%)	2.23 (1.56–3.37)
Cancer	4 (6.8%)	2.00 (1.71–2.32)
Primary immune deficiency	4 (6.8%)	1.80 (1.44–2.24)
Interstitial pneumonitis	3 (5%)	5.54 (1.51–12.7)
Cystic fibrosis	2 (3.4%)	4.49 (3.36–5.62)
Erdheim Chester disease	2 (3.4%)	3.05 (2.15–3.94)
Vasculitis	2 (3.4%)	3.68 (3.06–4.30)

IgG4-RD, IgG4-related disease. Apart from the final diagnostic retained, allergic and atopic manifestations were found in 10 patients (16.9%). As can be seen in Ebbo et al., there is a strong overlap in the different disease entities with respect to the serum levels of IgG4. This is why the IgG4 serum levels, if looked at in isolation, are not unambiguous in diagnostic terms. Both studies show non-IgG4-RD, but with markedly elevated IgG4 serum levels (Modified from [12, 13]).

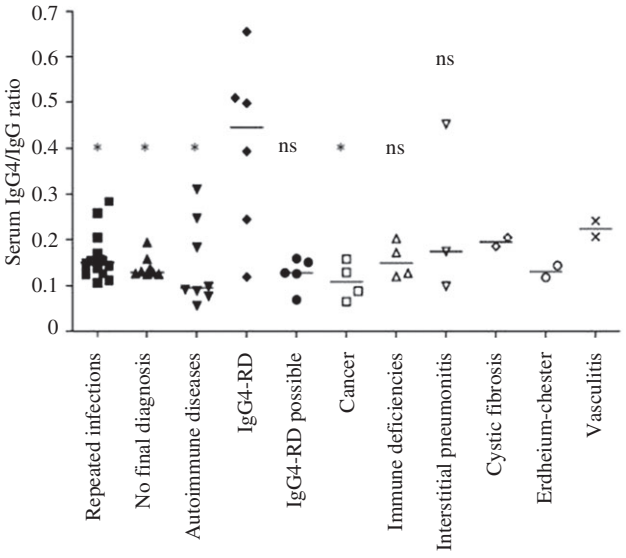

Figure 3 The ratio of serum IgG4/IgG-total taken on its own is not meaningful (modified from [12]).

This means that an increased IgG4 serum level is not specific for an IgG4-RD. Conversely, an IgG4-RD is relatively unlikely in connection with a IgG4 serum level in the normal range. Various studies have stated a sensitivity of 67%–95% and specificity of 90%–97% for elevated IgG4 serum levels in connection with IgG4-RD [16–21]. It is noteworthy that elevated IgG4 serum levels are found in about 5% of the healthy population [18, 22]. Elevated IgG4 serum levels are measured in 10% of patients even in the case of pancreatic cancer [18].

From the above it is clear that the diagnosis should always be confirmed by way of a biopsy.

The ratio of IgG4/IgG-total in the serum, too, shows overlapping values [12], so that it must be emphasized again that the IgG4 serum level is not suitable as a standalone parameter to confirm an IgG4-related disease; but if the patient has a standard IgG4 level, it can be largely ruled out (Figure 3).

Therefore, the following requirements apply to the diagnosis of an IgG4-RD [23]:

1. Clinical or imaging evidence of a fibrotic, tumor-like lesion at one or more organs
2. Histological confirmation

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Histopathology

According to a pathology consensus paper by an international commission [24], the quantitative extent of the IgG4+ plasma cell infiltration and the ratio between IgG4+ and IgG+ plasma cells are not by themselves decisive with respect to an IgG4-RD diagnosis. It is discussed there in detail that, depending on the disease duration and the affected organ, there may be severely fibrotic changes with very few inflammatory cells. Furthermore, in the case of very early lesions, fibrosis may be weak or absent, coupled with immense inflammatory cell infiltration.

The main histopathological features are characteristic fibrosis, inflammatory cell infiltration and phlebitis at varying stages in the affected organs, depending on the age of the lesion. The ratio of the IgG4+/IgG+ plasma cells >40% may be helpful, and may be indicative of a diagnosis if the histopathological features described below present themselves [25]. The histological features are listed in Table 6.

Insights into and views of IgG4-RD evolve quickly for clinicians. This is also true of the therapeutic approach, which will not be discussed extensively in this context. When this disease entity is suspected, clinicians, laboratory physicians and pathologists must work together closely in order to arrive at a correct diagnosis. In summary, the new pathological nomenclature for the diagnosis of IgG4-RD requires clinical organ swelling/tumor with serum IgG4 analysis (if elevated, it raises the probability; if not, it is not an exclusionary factor) as well as histological confirmation. IgG4-RDs can affect any organ and are not limited, as thought initially, to the pancreas.

Table 6 Histopathology of IgG4-related disease (modified from [24]).

	Inflammation	Fibrosis	Phlebitis	Others
Lacrimal gland	No unique features	Typical storiform fibrosis is relatively uncommon. More often collagenous fibrosis	Sometimes lacks obliterative phlebitis	
Salivary gland	Often associated with conspicuous lymphoid follicle formation	Storiform fibrosis is rare in parotid and minor salivary glands	Sometimes lacks obliterative phlebitis	
Lymph node	No unique features	Fibrosis is only seen in inflammatory pseudotumor-like lesions	Most often lacks obliterative phlebitis	Five histological patterns are recognized: (1) multicentric Cattleman's disease-like. (2) follicular hyperplasia, (3) interfollicular expansion, (4) progressive transformation of germinal center, and (5) nodal inflammatory pseudotumor-like. The specificity of these histologic changes in the absence of other evidence of IgG4-RD remains controversial
Lung	Small aggregates of neutrophils may be present in alveolar spaces or within the inflammatory infiltrates	Sometimes lacks storiform fibrosis, particularly in non-solid lesions (e.g. interstitial pneumonia)	No unique features	Obliterative arteritis is often seen in pulmonary manifestations, particularly solid lesions
Kidney	No unique features	No unique features	Obliterative phlebitis is less common particularly in needle biopsies	

Sjogren's syndrome

Laboratory diagnostics

A new ELISA test to detect IgA anti-M3 receptor autoantibodies holds out the promise of a possible future application in diagnosing Sjogren's syndrome [26].

Sjogren's syndrome is one of the most common autoimmune diseases [27]. Clinically speaking, the initial focus with Sjogren's syndrome is on the sicca symptoms of the mucous membranes, particularly of the eyes and mouth. The exact pathogenic mechanism of the disease is not yet clear. It is believed that damage to the salivary gland tissue activates T- and B-cells, including intensive infiltration of the salivary glands. This leads to the formation of autoantibodies against SS-A, SS-B and α-fodrin.

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It has further been shown that antibodies against the external region of the muscarinic 3 receptor can block the production of saliva. The salivary glands are innervated intensively by nerve fibers of the parasympathetic nervous system and stimulated by the neurotransmitter acetylcholine. Overall, patients with primary Sjogren's syndrome frequently exhibit autonomic dysfunction with a marked fatigue problem [28], possibly as a result of the interaction of anti-muscarinic receptor antibodies at the sympathetic and parasympathetic nervous systems. At least the treatment with muscarinic 3 receptor antagonists, such as pilocarpine, will increase secretion in patients whose salivary glands have not yet been fully destroyed.

Given the pathophysiological significance of muscarinic 3 receptors, as explained, several working groups have been set up to study the detection of anti-muscarinic 3 receptor antibodies. The data regarding the detectability of IgG autoantibodies and their significance in the diagnosis of Sjogren's syndrome are heterogeneous in the literature [29–31]. Antibody diagnostics is of great importance to differentiate the more common, non-autoimmune sicca symptoms in the population [27] and the autoimmune pathogenesis in connection with Sjogren's syndrome.

Li et al. [26] have developed a new ELISA test for the detection of the IgA instead of IgG anti-muscarinic 3 receptor (second loop of the extracellular region). Subsequently, the ELISA test was validated in a group of patients with primary and secondary Sjogren's syndrome, systemic lupus and rheumatoid arthritis, as well as healthy individuals. Table 7 shows the sensitivity and specificity of the ELISA test in connection with the various autoimmune diseases. The test does not provide for effective differentiation between the autoimmune diseases examined. However, the IgA antibody is rather rare in healthy individuals.

By combining it with the antibodies SSA and SSB established for Sjogren's syndrome, it was possible to calculate a significantly higher sensitivity, with the specificity remaining relatively unchanged, in the ROC analysis.

Table 7 IgA anti-muscarinic receptor 3 sensitivity and specificity in inflammatory rheumatic diseases (modified from [26]).

Rheumatic diseases	n	Sensitivity (%)	Specificity (%)
pSS	91	42/91 (46)	129/149 (87)
sSS	16	6/16 (38)	168/224 (75)
SLE	27	5/27 (19)	156/213 (73)
RA	40	6/40 (15)	144/200 (72)
Normal	66	3/66 (5)	–

pSS, primary Sjogren's syndrome; sSS, secondary SS; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis.

Table 8 Sensitivity and specificity of antibodies in Sjogren's syndrome (modified from [24]).

Antibodies	Sensitivity (%)	Specificity (%)
Anti-c2M3RP	46	87
Anti-SSA	56	91
Anti-SSB	24	97
Anti-SSA or anti-c2M3RP	71	85
Anti-SSB or anti-c2M3RP	55	89
Anti-SSA or anti-SSB or anti-c2M3RP	73	84

Here, too, similar to the MBDA score for rheumatoid arthritis, a combined test has better diagnostic properties than any parameter on its own (Table 8). This may well be an important factor when it comes to considering a complex pathophysiological event in connection with autoimmune diseases. A single marker, therefore, is only of limited significance, because it can only represent a reflection of one possible facet of the disease.

Conflict of interest statement

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