Novel autoantibodies in rheumatoid arthritis

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SUMMARY

Rheumatoid factor and antibodies against cyclic citrullinated peptides represent a diagnostic hallmark in rheumatoid arthritis (RA). However, over the last decades many other autoantibodies have been identified. Several proteins can trigger an aberrant autoimmune response in their native form while others acquire this feature after post-translational modifications such as citrullination, carbamylation or acetylation. It is of interest that also the enzymes catalyzing such post-translational modifications (*e.g.* the protein arginine deiminases) can transform themselves into autoantibodies in RA. The purpose of this review article is to provide an overview of relevant literature published over the last years regarding novel autoantibodies and their possible diagnostic and prognostic significance in RA.

Key words: *Rheumatoid arthritis; Autoantibodies; Citrullination.*

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INTRODUCTION

Rheumatoid arthritis (RA) is the most common and heterogeneous chronic inflammatory joint disease primarily affecting synovial joints. It is characterized by erosive arthritis, extra-articular manifestations, disability and mortality, the latter mainly due to cardiovascular events and respiratory complications (1).

Although the exact cause of the disease is still unknown, the identification of a number of etiopathogenic features, such as the presence of auto-antibodies (Abs) directed against citrullinated proteins (ACPA) and the increased production of inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-17 (2), has led in recent years to substantial innovations in both diagnosis and treatment of the disease and consequent improvement of the prognosis. It is now evident, in fact, that early diagnosis and aggressive treatment with conventional synthetic (cs) and biologic (b) disease modifying anti-rheumatic drugs (DMARDs) in patients with RA may prevent irreversible bone loss and life-time disability (3). Although Abs against many different epitopes are continuously identified in the serum of RA patients, actually only 2 types of autoAbs, rheumatoid factor (RF) and Abs directed against synthetic cyclic citrullinated peptides (anti-CCP), are recognized as having a diagnostic and prognostic value and have been included in the 2010 American College of Rheumatology (ACR) RA classification criteria (4, 5).

RF, an Ab recognizing the Fc or conserved portion of human Abs, was the first biological criterion included in the ACR criteria of 1987 (4), showing a great sensitivity for established RA and allowing identification of patients with persistent and/or erosive disease. It was, therefore, the first recognized prognostic marker in RA. However, RF presents two main disadvantages: low diagnostic specificity, since it could be also detected in other autoimmune diseases, infectious disorders, malignancies, other connective tissue diseases and in a proportion of healthy controls, and low prevalence in the initial phases of the disease (6).

ACPA are the second biological criterion introduced in the 2010 ACR/EULAR RA classification (5), as they display a higher specificity, could be detectable also in the pre-clinical and early stages of the disease and correlate with erosive disease (6).

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In this review, we provide an overview of the past 5 years of scientific literature about novel autoAbs and their possible diagnostic, clinical, prognostic and predictive significance in RA.

CITRULLINATED PEPTIDES

Citrullination is a post-translational modification catalyzed by the enzyme peptidylarginine deiminase (PAD), leading to the conversion of arginine residues into the non-standard amino-acid citrulline. This process determines the loss of a positive charge with a very small change in molecular mass and can influence the ability to form hydrogen bond and therefore the interaction with other amino-acids residue. Citrullination leads to the creation of a new protein with conformational and possibly functional alteration but, most importantly, it leads to the formation of new immunogenic epitopes, eventually triggering an aberrant immune response in genetically predisposed individuals (7).

Anti-perinuclear factor (APF) (8) and anti-keratin antibodies (AKA) (9) were described for the first time in RA patients in 1964 and 1979 respectively, but only in 1998 did Schellekens et al. (10) identify citrullinated pro-filaggrin as the target of these 2 autoAbs. This was the first evidence of the importance of citrullination in the pathogenesis and immunologic response of RA.

ACPA entered the clinical routine after the development of ELISA tests based on the recognition of anti-CCP (11) showing great specificity and good sensitivity. Nowadays second generation anti-CCP2 assays are used in clinical practice, raising the specificity around 96% with a sensitivity that ranges from 67% to 78% in patients with established RA and 57% in patients with early RA (12). ACPA can be detected in early RA and in RF-negative patients and are associated with more severe joint destruction (13).

CCP are not the real epitopes recognized by ACPA, as many citrullinated proteins seem to be the actual target of these Abs and numerous assays detecting these epitopes have been developed in the past years (Table I).

Vimentin: anti-mutated citrullinated vimentin/anti-Sa

One of the most important citrullinated antigen targets of ACPA is vimentin, an intermediary filament fundamental for cell

Table I - ACPA fine specificities epitopes and related antibodies.

Epitopes	Antibodies	Sensitivity	Specificity	Reference
Pro-filaggrin / Keratin	AKA	48%	98%	(16)
	APF	-	-	-
	Anti-fil 306-326	42%	95%	(17)
	Anti-fil 311-315	72%	95%	(17)
	Anti-keratin 8	68%	80%	(29)
Vimentin	Anti-MCV	39-79%	73.8-100%	(16-18)
	Anti-Sa	37-50%	97-99%	(19, 20)
Alpha-enolase	Anti-CEP1	44%	97%	(39)
Fibrinogen/Fibrin	Anti-FibA	48%	95%	(32)
	Anti-β60-74Cit$_{60,72,74}$	70-73%	95%	(32, 33)
	Anti-α36-50Cit$_{38,42}$	45-50%	95%	(32, 33)
Viral peptides	Anti-EBNA35-58Cit	36%	98%	(35)
	Anti-VCP2	61%	95%	(23)
Glucose-6.phosphate isomerase	Anti-GPI	75%	64%	(16)
	Anti CCG (1-9)	-	-	(39)
Collagen	Anti-telopeptides type I	47%	96%	(24)
	Anti-telopeptides typeII	41%	94%	(24)
	Anti-type II collagen	30%	-	(17)

Table II - Sensitivity and specificity of antibodies directed against vimentin-derived peptides.

Author	Year	N° patients	Sensitivity	Specificity	Reference
Anti-MCV					
Reyes-Castillo et al.	2015	170	61%	100%	(18)
Gonzalez-Lopez et al.	2014	225	70%	N/A	(26)
Zhu et al.	2013	41	79%	74%	(16)
Nicaise-Roland et al.	2013	592	39%	96%	(21)
Bartoloni et al.	2012	285	59%	95%	(23)
Szarka et al.	2013	263	44%	95%	(17)
Anti-Sa					
Hou et al.	2012	198	50%	99%	(20)
Iwaskiewicz et al.	2015	41	37%	97%	(19)

structure, and Abs directed against vimentin may be among of the first to develop in the preclinical phase of the disease. To date, two ELISA assays to detect anti-vimentin Abs exist, the first directed against citrullinated vimentin (named after a patient as anti-Savoie/anti-Sa), the second directed against mutated citrullinated vimentin (MCV), a peptide with insertion of a glycine residue instead of an arginine residue (14, 15).

The sensitivity of anti-MCV Abs in RA patients ranges from 39% to 78.6% and the specificity between 74% to 100% (16-18), while anti-Sa Abs display lower sensitivity (37%-50%) but higher specificity (97-99%) (19, 20) (Table II).

The presence of Abs directed against MCV strongly correlates with the presence of anti-CCP Abs (19-21). An interesting concordance has been also found between anti-Sa Ab status and presence/titer of Abs directed against carbamylated proteins (22). Some studies, comparing the diagnostic value of anti-MCV and anti-CCP Abs, did not find further diagnostic information from the addition of anti-MCV to anti-CCP detection (18, 23). Only Zhu et al. (16) found that the combined detection of anti-MCV and anti-CCP Abs increased the sensibility to 89% compared with anti-CCP alone (68%) and anti-MCV alone (79%), but with a loss of specificity of anti-CCP from 98% to 74% in a small cohort of RA and mixed connective tissue disease (MCTD) patients.

An interesting data point is also that the presence of anti-MCV autoAbs in patients with undifferentiated arthritis (UA) is correlated with the development of RA with a positive predictive value (PPV) of 72.5% (21).

Anti-MCV and anti-Sa Abs also seem to be associated with disease activity, since a statistically significant direct correlation was found with disease activity score on 28 joints (DAS28) (18, 20), and with development of erosive disease (24).

A positive correlation was found also between anti-MCV Abs, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and disease duration (18), whereas this association was not confirmed for anti-Sa Abs (19, 20). Conflicting results concerning the association between these autoAbs and radiological articular damage have been recently reported (19, 20, 24).

Reyes-Castillo et al. (18) found a strong correlation between the presence of anti-MCV Abs and Th1 (TNF-α, IL-1β) and Th2 (IL-4, IL-6, IL-10, IL-2) related cytokines levels, but not with IL-17, in a cohort of 170 RA patients. Furthermore, a relationship between the homozygous susceptibility haplotype of PADI4 gene and levels of anti-MCV Abs has been also described.

A strong association between anti-MCV levels and extra-articular manifestation of RA, in particular Felty's syndrome and major cutaneous vasculitis, has been found in a small cohort of RA patients (25). Another study, examining the possible association

between anti-MCV and extra-articular RA manifestations, detected only a weak association with rheumatoid nodules (26).
Anti-MCV seem to be also a predictive marker of response to rituximab (RTX) treatment as patients responding to this biological agent were characterized by a restrictive Ab response of anti-MCV IgG, while double positivity for anti-MCV IgG and IgA was associated with response failure to RTX (27).

Filaggrin and keratin: the anti-keratin Abs
Keratin and filaggrin, two intermediate filaments, were the first two epitopes recognized as targets of autoAbs in RA patients, the so called AKA, that were first described in 1979 in RA serum (9). One of the targets of these Abs was later identified as citrullinated pro-filaggrin (28).
Recently, AKA showed the highest specificity (98%), but the lowest sensitivity (48%), in a diagnostic comparison with anti-MCV Abs and anti-glucose-6-phosphate isomerase (GPI) Abs (16).
Abs against keratin 8 (K8) were identified in a small group of 50 patients with RA with a sensitivity of 68% and a specificity of 80% and K8 was probably one of the real targets of the traditional AKA (29). Data obtained with a Multipin ELISA analysis showed that two citrullinated filaggrin fragments, the fil306-326 and its minimal epitope fil311-315, were recognized by RA sera with a sensitivity of 42% and 72%, respectively, but a high specificity (95%) (17).

Fibrin and fibrinogen: anti-hFibA Abs
Citrullinated fibrin was identified as one of the main ACPA autoantigens in the synovial tissue of RA patients (30) and an ELISA test was developed to detect Abs to human citrullinated fibrinogen (hFibA) (31).
Anti-hFibA Abs showed a sensitivity of 48% and a specificity of 95% in RA patients and a sensitivity of 32% with a specificity of 88% in predicting the progression to RA in a cohort of patients affected by UA. Disability, as quantified by the health assessment questionnaire (HAQ), and disease activity (DAS28) were directly correlated with the presence of anti-hFibA Abs (21).
In a cohort of established RA patients, Abs directed against 2 fibrin epitopes β60-74Cit$_{60,72,74}$ and α36-50Cit$_{38,42}$, showed a sensitivity of 70% and 50% respectively (diagnostic thresholds specificity of 95%). An interesting piece of data was that 90% of the RA sera with circulating anti-hFibA Abs contained one or both the subfamilies of autoAbs (32).
In a cohort of black-African RA patients, anti-hFibA Abs displayed a sensitivity of 73%, and, similarly to Cornillet's study (32), Abs against α36-50Cit$_{38,42}$ and β60-74Cit$_{60,72,74}$ were detected respectively in 45% and 73%. Anti- β60-74Cit$_{60,72,74}$ Abs had also a statistically significant association with HLA-DRB1 SE (33).

Viral citrullinated peptide
Involvement of the Epstein Barr Virus (EBV) in RA pathogenesis was suggested when Abs recognizing a nuclear antigen of EBV-transformed lymphocytes were found in RA sera. The antigen target of these Abs was initially called RA nuclear antigen (RANA) and later identified as the EBNA-1 viral protein (34).
EBNA35-58Cit is a synthetic citrullinated multiple antigen peptide bearing four identical sequences derived from the EBNA-1 region of the genome of EBV. Abs directed against this epitope were found in 36% of established RA patients with a specificity of 98%, and were directly correlated with the presence of anti-hFibA, thereby suggesting a cross-reactivity between these two autoAbs (35).
Viral citrullinated peptide 2 (VCP2), a peptide corresponding to the sequence 338-358 of the EBV encoded protein (EBNA-2), is another target of specific Abs in RA sera. The diagnostic performance of anti-VCP2 Abs was compared with anti-MCV in a large cohort of RA patients, revealing a similar sensitivity (61% and 59% respectively), but higher specificity for anti-VCP2 (95% *vs* 92%) (23).

Collagen and telopeptides
Collagen is the main component of connective tissue. Abs directed against collagen type II are present in about 30% of RA patients and correlate with HLA-DR4

(36). Abs against citrullinated collagen II peptide showed a sensibility of 41% and a specificity of 95% (17).
In addition, Abs recognizing citrulline-containing sequences related to type I and II collagen telopeptides can be found in sera of RA patients with a sensitivity of 47% and 41% and a specificity of 96 and 94% respectively (24).
Finally, Abs reactive against citrullinated-carboxy-terminal telopeptides of type I and type II collagen have been observed in both seronegative and seropositive RA patients, even in samples collected before the development of the disease, and their titer was higher compared to controls (37).

Alpha-enolase 1: anti-CEP1
Alpha-enolase 1 is a glycolytic isoenzyme that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate. Abs directed against citrullinated alpha-enolase 1 (CEP1) were shown to be a portion of ACPA fine specificity in RA patients (38).
Anti-CEP1 Abs were detected in 44.2% of RA patients with a specificity of 97.3% and were associated with HLA-DRB1 SE alleles in a Japanese cohort (39). Furthermore, an association between anti-CEP1 and PTPN22 has been also reported (40, 41).
In another cohort of 248 RA patients, anti-CEP1 and anti-P. Gingivalis Ab levels were higher in RA patients than in controls and correlated each other. Moreover, anti-CEP1 also correlated with periodontal disease and RA disease activity (42). Conflicting data, however, have been reported with regard to the association of CEP1 and erosive RA (41, 43).

Glucose-6-phosphate isomerase: anti-GPI
Glucose-6-phosphate isomerase (GPI) is another major glycolytic enzyme and was first described as an arthritogenic target in a mouse model, in which arthritis was sustained almost completely by autoAbs to GPI (44).
Anti-citrullinated-GPI Abs have been recently identified for the first time in the sera of RA patients. In particular, Abs against 9 cyclic citrullinated peptides spanning the whole GPI sequence (CCG 1-9) have been described by Umeda *et al* (39). Anti-CCG-2 and anti-CCG-7 Abs were highly specific for RA, their titers correlated with the presence of HLA-DRB1 SE alleles and with RA disease activity. Furthermore, the treatment with TNF antagonists was able to reduce their titers.
In a study comparing ACPA fine specificities, Zhu et al. found that anti-GPI had the lowest specificity (64%) and a sensitivity of 75% compared with anti-CCP, anti-MCV, anti-AKA (16).

Novel citrullinated targets
Van Beers et al. (45) identified new citrullinated epitopes as targets of the immune system in RA, including citrullinated apolipoprotein E (ApoE), myeloid cell nuclear differentiation antigen (MNDA) and β-actin. These Abs were detected in the sera of patients with established RA with a sensitivity of 27%, 16%, 27% and a specificity of 96%, 97%, 98%, respectively.
The great interest towards Abs targeting citrullinated peptides led to a consistent bulk of data that, however, needs to be validated in larger studies. Some Abs including anti-MCV seem to be a predictive marker of progression to RA in UA patients (21-37), and some anti-MCV isotypes seems to be associated with extra-articular manifestations (25) or the response to rituximab (27).
Overall, in our opinion these data show no real clinical benefit for the assessment of a single epitope Ab instead of anti-CCP, as their sensitivity and specificity seems to be quite similar or even lower compared to anti-CCP (Table I). Likewise, the correlation with disease severity of these novel Abs appears to overlap that of anti-CCP.
Conflicting results have been reported regarding the relevance of the detection of multiple specificities Abs in RA diagnosis in comparison to anti-CCP alone (16-21). However, additional data are needed to draw definitive conclusions in this regard.

PEPTIDIL-ARGININE DEIMINASE (PADS)

Besides citrullinated proteins, it is interesting to note that also PADs, the enzymes

catalyzing citrullination, may represent a target antigen for autoAbs in RA. PADs are calcium-dependent enzymes and five isoforms have been described in humans (1-4 and 6). In particular, PAD3 and PAD4 emerged as key participants in the pathogenesis of RA (46) and targets of autoAbs in RA sera (47). Among all the isoforms, PAD4 seems to be the most relevant target. Anti-PAD4 sensitivity ranges from 24% to 37% (18, 48-51), while specificity, determined only in two studies, appears to reach 95-100% (18, 51). Anti-PAD4 Abs have been directly correlated with disease duration (18) and with the presence of anti-CCP Abs (18, 48-51). Conversely, no association was found between anti-PAD4 Abs and Th2-Th1 cytokines levels (18) or HLA DRB1-SE (51). It is worth noting that patients with anti-PAD4 Abs also had negligible levels of serum human PAD4 (48).
PAD3 is another recognized target of serum autoAbs in RA patients and anti-PAD3 sensitivity varied between 11% and 18%. Similarly to anti-PAD4, the presence of anti-PAD3 Abs was associated with the presence of anti-CCP Abs (50, 52).
It has been shown that patients with anti-PAD3 Abs had a higher baseline Sharp van der Heijde (SvdH) score than those with anti-PAD4 alone. In addition, anti-PAD3 Abs were also associated with longer disease duration, radiographic progression and erosive disease (50, 52). Finally, the presence of anti-PAD3/4 cross-reactive antibodies seemed to be associated also with the presence and the extent of interstitial lung disease (ILD) assessed by computed tomography (CT) scan (50).

CARBAMYLATED PROTEINS

Carbamylation is a post-translational process in which cyanate binds to primary amino or thiol groups. This process is enhanced by inflammation via a mechanism, which depends on myeloperoxidase (MPO) and lysine carbamoyltransferase, the enzymes that catalyze the carbamylation of a lysine residue into homocitrulline (53).
Carbamylation is involved in many pathological processes including chronic kidney disease, cardiovascular disease and cataract, but it is interesting to underline that Abs to carbamylated proteins (Carbp), in particular carbamylated-fetal-calf-serum (Ca-FCS) and carbamylated-fibrinogen (Ca-Fib), have been identified in the sera of RA patients (54).
These Abs have been observed in the sera of patients years before disease onset (55, 56) and their presence in patients with UA (57) or arthralgia (58, 59) was significantly associated with future development of RA independently of other ACPA and RF status. The reported sensitivity of anti-Carbp Abs in RA is 18-26% in patients prior to the diagnosis, and 27-46% in patients with overt RA, while the specificity varies between 89% and 97%. (22, 55-63) (Table III).
There is evidence that positivity for anti-Carbp Abs is also associated with the presence of anti-CCP and anti-Sa and correlates with the titers of these Abs (22). Likewise, only a small percentage, 6.1-29.5%, of seronegative (CCP, RF) RA patients were positive for anti-Carbp Abs (22, 55, 58, 59), and 11% of the sera of seronegative patients collected before the diagnosis were tested positive for anti-Carbp Abs (56).
Anti-Carbp Abs were also directly correlated with disease activity (DAS28), disability (HAQ) and the presence of joint erosions (60, 62).
Interestingly, the presence of anti-Carbp Abs before the onset of disease symptoms predicted the radiological findings at baseline and correlated with the rate of radiological destructions (55).
In contrast to these results, no correlation between anti-Carbp Abs and DAS28, radiological damage, ESR or PCR levels was found by Challener et al. (22).
With regard to genetic factors, in a retrospective study on two cohorts of patients, Jiang et al. (61) found no correlations between the presence of anti-CarbP Abs and PTPN22 polymorphism or HLA-DRB1-SE alleles. The same study ruled out the association between anti-Carbp Abs and smoking. Data about anti-CarbP Abs are nowadays consistent and allow us to make some considerations. The main clinical relevance of anti-Carbp Abs is represented by their di-

Table III - Sensitivity and specificity of anti-Carbp antibodies.

Author	Year	N° Pts	Sensitivity	Specificity	Reference
Brink et al.	2015	423	42% (RA patients) 19% (Prior diagnosis)	97%	(55)
Gan et al.	2015	76	26% (Prior diagnosis)	95%	(56)
Yee et al.	2014	120	30%	No controls	(62)
Shi et al.	2014	79	27% (for anti-Ca-FCS) 38% (for anti-Ca-Fib)	No data	(63)
Jiang et al.	2014	1985+846	36-45% (for anti-Ca-FCS) 38-43% (for anti-Ca-Fib)	No data	(61)
Challener et al.	2016	212	46% (seropositive RA patients) 9% (seronegative RA patients)	No data	(22)
Shi et al.	2015	969	44%	89%	(57)
Pecani et al	2016	309	46.8% 29.5% (seronegative RA patients)	91.95%	(59)

agnostic utility in seronegative (RF, CCP) RA patients. Indeed, a proportion of RA patients ranging between 6.1% and 29.5% (22, 55, 58, 59) may benefit from the assessment in the diagnostic workout. Nevertheless, the seronegative gap is yet to be filled and further research is needed in this regard. Although these Abs display a lower sensitivity compared to anti-CCP, their correlation with the development of RA in UA and arthralgia patients appears to be of great interest.

OTHER TARGETS

The identification of novel targets of autoAbs in RA is under intense investigation. Therefore, a wide range of autoAbs, other than ACPA and anti-Carbp, are continuously identified in RA patients (Table IV).
Abs against reactive oxygen species (ROS) modified type II collagen (CII) may represent a novel serologic diagnostic marker in RA. The reactivity to ROS-CII in DMARDs naive patients with early RA was significantly higher than in patients with OA and healthy control subjects. A different reactivity was also found in patients with established RA between DMARD-non-responder patients (sensitivity 58%) and DMARD-responders (8%) (64). No relationship has been observed between anti-ROS CII and DAS28.
Serum levels of Abs directed against N-homocysteinylated (Hcy) albumin and haemoglobin were elevated respectively in 21% and 22% RA patients compared to controls. In addition, a significant relationship between anti-N-Hcy-proteins Abs and RA duration, radiological damage and number of swollen joints was observed (65).
14-3-3η, a novel proinflammatory mediator, involved in the pathophysiology of joint inflammation in RA, seems to be able to generate an Ab response as Abs against this compound have been identified in RA. The titers of these Abs were significantly higher in patients with early RA when compared with established RA patients and healthy controls, and 72% of the seronegative (CCP, RF) early RA patients were positive for anti-14-3-3η Abs. Interestingly anti-14-3-3η autoAbs were significantly lower at year 1 in comparison to the corresponding baseline measurement in a cohort of 62 early RA, DMARDs naïve patients, receiving therapy within a year of the first assessment (66).
Heterogeneous nuclear ribonucleoproteins (hnRNPs) are nucleoplasmic molecules interacting with pre-messenger ribonucleic acid. Abs reactive with hnRNP-B1, also called RA33, were observed in Russian patients with rheumatic diseases, in particular systemic sclerosis and RA (sensitivity 14%) and, in the latter, they were not associated with disease activity or erosions (67). A lower sensitivity of anti-RA33

Table IV - Novel autoantibody target of in RA.

	Authors	Year	Reference
ROS modified type II collagen	Strollo et al.	2013	(64)
N-homocysteinylated albumin and haemoglobin	Nawakowska-Plaza et al.	2014	(65)
14-3-3η	Maksymowych et al.	2015	(66)
Heterogeneous nuclear ribonucleoproteins (hnRNPs)	Maslyanskiy et al. Al-mughales et al.	2014 2015	(67) (68)
Heat shock proteins (HSP)	Shoda et al.	2016	(69)
α 1,4-polygalacturonic acid (PGA)	Dai et al.	2014	(70)
Osteoprotegerin (OPG)	Hauser et al.	2015	(71)
Nuclear phosphoprotein IFN-inducible protein 16 (IFI-16)	Alunno et al.	2016	(72)
Tumor necrosis factor (TNF)	Lopatnikona et al.	2013	(73)
Malondialdehyde-Acetaldehyde	Thiele et al.	2015	(74)
Transthyretin (TTR)	Sharma et al.	2014	(75)
Tryptase	Guo et al.	2014	(76)
Carbonic anhydrases III (CA)	Liu et al.	2012	(77)
Vascular endothelial Cadherin	Bouillet et al.	2013	(78)
Glial fibrillary acidic protein (GFAP)	Biswas et al.	2013	(79)
α-1-β-glycoprotein (A1BG)	Biswas et al.	2013	(79)
Zinc finger protein 706 (ZNF706)	Charpin et al.	2013	(80)
Within BGCN homolog drosophila (WIBG)	Charpin et al.	2013	(80)
Gaba A receptor-associated protein-like 2 (GABARAPL2)	Charpin et al.	2013	(80)

(7%) was observed in another RA cohort from Saudi Arabia (68).

Heat shock proteins (HSP) are molecular chaperones required for cell homeostasis. Abs against Mycobacterial HSPs were significantly elevated in RA patients and correlated with serum anti-binding immunoglobulin protein (BIP) Ab titers (69).

α1,4-polygalacturonic acid (PGA) is a major structural component of pectin. In a cohort of 100 RA patients, when diagnostic specificity was set at 95%, PGA-IgA and PGA-IgG Abs were detected in 65% and 77% of RA patients, respectively (70). In this study, the sensitivity of PGA-IgA Abs was 70% in early RA subjects.

AutoAbs to bone protective protein osteoprotegerin (OPG) were identified in 9% of RA patients and were associated with disease activity and increased bone resorption (71).

IFN-inducible nuclear phosphoprotein 16 (IFI-16) is a member of the HIN-200/Ifi-200 gene family whose overexpression drives early steps of inflammatory response through NF-kb-mediated secretion of pro-inflammatory molecules. Circulating anti-IFI16 autoAbs were identified in approximately 20% of RA patients (72).

Notably, circulating anti-TNF Abs were higher in the sera of RA patients compared to controls. In particular, IgG2, IgG3, IgG4 anti-TNF Abs were significantly higher in patients with active RA than in healthy controls, IgG2 were also significantly higher in sera of patients with active disease than in patients who had responded positively to DMARDs therapy (73).

Other epitopes were recognized as targets of autoAbs in RA like malondialdehyde-Acetaldehyde (MAA) (74), transthyretin (TTR) (75), tryptase (76), carbonic anhydrases III (CAIII) (77), vascular endothelial cadherin (78), but no additional information is available at the moment.

Finally, through mass spectrometric analysis, Biswas et al. (79) identified two novel targets of autoAbs, glial fibrillary acid protein (GFAP) and α-1-β-glycoprotein

(A1BG). Moreover, Charpin et al. (80) identified zinc finger protein 706 (ZNF706), within BGCN homolog drosophila (WIBG), and gaba A receptor-associated protein-like 2 (GABARAPL2), as new targets of RA serum autoAbs.

CONCLUSIONS

The identification of novel serum biomarkers in heterogeneous and disabling diseases such as RA is helpful nowadays, not only for an early diagnosis, but also for a prognostic stratification of the patients. In fact, the early identification of subjects at higher risk of developing more aggressive forms of the disease would provide the opportunity to start a treatment before the onset of permanent damage and disability and ideally to prevent, or at least slow down, their development. Although some autoAbs already represent a reality as diagnostic and prognostic markers in RA, the identification of novel targets of immune response in RA would helpfully allow closure of the gap of seronegative RA and ensure early diagnosis, early prognostic stratification and prompt tailored treatment with a treat-to-target strategy for each patient.

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