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Susceptibility of Vitamin D Receptor SNP (C>A rs7975232) to be a risk factor for incidence of rheumatoid arthritis(RA)

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Abstract

- one of a chronic inflammatory important is Rheumatoid arthritis(RA) illness that degrades cartilage and, eventually, causes bone erosion and disability. Rheumatoid arthritis, an autoimmune disorder, occurs when your immune system mistakenly attacks your body's own tissues. This ailment has been given the moniker "Objective:" for whatever reason. The fundamental target of this research was to investigate the significance of the VDR SNP (rs7975232) in rheumatoid arthritis patients from Iraq, specifically with regard to fatty acid synthase (FAS) and interleukin-17A. Various Methods: In this study, the demographic information of the participants who had rheumatoid arthritis (RA) and the participants who were healthy served as controls. There were a total of 45 participants with RA and 45 healthy persons. The findings of the study demonstrate that the levels of total cholesterol and LDL cholesterole in the individuals increased significantly (p0.0001 and p0.001, respectively), whilst the levels of HDL cholesterol declined significantly (p0.001 and p0.1, respectively). But neither TG nor VLDL altered in a way that was statistically significant over this time period. According to the findings of the current investigation, there is a statistically significant variance between the levels of the faty acid synthase FAS enzyme in the patient's group (0.93 ± .0. 53) and the levels in the control group (0.74 ± o.45), with a p_ value of 0.001. Comparing the two different sets of values led us to this conclusion. The findings of this research indicate that there is a statisticaly significant gap, with a p_ value of 0.001, between the levels of IL-17A (pg//ml) found in the patients(166/13)and the controle group (87/5). The assessment of both FAS and IL_ 17A has been recommended as a style that is both practicable and helpful for the primary diagnosis of RA patientes in addition the continues tracking of those affected by this disease.

Keywords: Rheumatoid arthritis, vitamin D receptor, Fatty acid synthase, IL-17A, lipid profile

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قابلية مستقبل فيتامين د (SNP (C> A rs7975232 ليكون عامل خطر للإصابة بالتهاب المفاصل الروماتويدي (RA)

شيماء العبيدي[1] ، أسماء محمد مكي[2] ، سرى عوض [3]

المستخلص

أحد الأمراض الالتهابية المزمنة هو مرض التهاب المفاصل الروماتويدي (RA) الذي يؤدي إلى تدهور الغضروف، وفي النهاية يسبب تآكل العظام والإعاقة. يحدث التهاب المفاصل الروماتويدي، وهو اضطراب في المناعة الذاتية، عندما يهاجم جهازك المناعي عن طريق الخطأ أنسجة الجسم. لقد أُطلق على هذا المرض لقب "الهدف:" لأي سبب كان. كان الهدف الأساسي من هذا البحث هو دراسة أهمية VDR SNP (rs7975232) في مرضى التهاب المفاصل الروماتويدي من العراق، وتحديداً فيما يتعلق بسينسيز الأحماض الدهنية (FAS) والإنترلوكين-A17. طرق مختلفة: في هذه الدراسة، كانت المعلومات الديموغرافية للمشاركين الذين يعانون من التهاب المفاصل الروماتويدي (RA) والمشاركين الأصحاء

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بمثابة عناصر تحكم. كان هناك ما مجموعه 45 مشاركا مع RA و 45 شخصا أصحاء. أظهرت نتائج الدراسة أن مستويات الكوليسترول الكلي وكوليسترول LDL لدى الأفراد زادت بشكل ملحوظ (p0.0001 وp0.001، على التوالي)، في حين انخفضت مستويات الكولسترول HDL بشكل ملحوظ (p0.001 وp0.1، على التوالي).). لكن لم يتغير أي من TG أو VLDL بطريقة كانت ذات دلالة إحصائية خلال هذه الفترة الزمنية. وفقا لنتائج التحقيق الحالي، هناك تباين ذو دلالة إحصائية بين مستويات إنزيم FAS سينسيز الأحماض الدهنية في مجموعة المريض (0.93 ± 0.53.) والمستويات في المجموعة الضابطة (0.74 ± o.45))، بقيمة p_ تبلغ 0.001. إن المقارنة بين المجموعتين المختلفتين من القيم قادتنا إلى هذا الاستنتاج. تشير نتائج هذا البحث إلى وجود فجوة ذات دلالة إحصائية بقيمة p_0.001 بين مستويات IL-17A (pg//ml) الموجودة في المرضى (13/166) ومجموعة التحكم (87/ 5). تمت التوصية بتقييم كل من FAS و IL_ 17A كأسلوب عملي ومفيد للتشخيص الأولي لمرضى التهاب المفاصل الروماتويدي بالإضافة إلى التتبع المستمر للمتضررين من هذا المرض

الكلمات المفتاحية : التهاب المفاصل الروماتويدي، مستقبلات فيتامين د، انزيم تخليق الأحماض الدهنية، انترلوكين17أ، مستوى الدهون

Introduction

Rheumatoid arthritis, so known simply as RA, is a degenerative autoimmune condition that effects on the joints over time. It is characterized by progressivee, symmetrical inflamnation of affected joints, eventualy leading to cartilage loss, bone erosione, and disability [1].In the beginning, the disease affects a relatively limited number of joints; however, as the disease progresses, a greater number of joints are injured, and extra-articular symptoms become more prevalent [2].When compared side by side, the symptoms of RA in its early stages and those of the illness in its later, untreated phases are markedly distinct from one another from a clinical perspective. In the early stages of rheumatoid arthritis, symptomes such as fatiguee, flu-.like symptoms, swollen and Joint pain, morning stiffness, highly C-reactive protein (CRP) and erythrocyte sedimentation rat (ESR) are all indicators o[3]f the condition. Testing for elevatd levels of CRP and ESR can be helpful in making an early diagnosis of Rheumatoid arthritis (RA). Studies have revealed that both genetic and environmentale variables have important role in progressive of the disease [4], even if the precise genesis of RA is still a mystery and has not been discovered. Smmoking, obesity, exposuree to ultraviolet radiation, sexual hormones, drug use, changes in the microorganisms of the gutt, mouth, and lungs, periodontal disease, and infections are whole known to increase the risk of rheumatoid arthritis[5-8]. The synthesis of IL _ 17A by Th17 cells encourages the migration of neutrophils and the make and production of pro-inflammatory cytokiness such as IL_6, IL_8, and GM_CSF by epithelial, endothelial, and fibroblastic cells [9].This results in inflammation in the region that is afflicted, which has the effect of hastening the advancement of the disease. As a consequence of the acts that IL-17A is responsible for, RA patients experience a worsening of bone erosion, cartilage degradation, and ne0angiogenesis [10] . In addition to this, it has been demonstrated that IL-17A promotes synovi0cytes to create matrix metalloproteinase (MMP)-1, which ultimately results in the destruction of cartilage [11]. Research has shown that the development of RA is associated with a crucial mechanism known as angiogenesis. Within the framew0rk of this discusion, it has been dem0nstrated that IL_17A promotes the migrattion of endotheliale cells [12]and the synthessis of vascular endothelial growth fact0r (VEGF) by syn0vial fibroblasts

[13].The human genome has a gene called FASN that is responsible for coding for the fatty acid synthease (FAS)enzyme [14] .The FAS protein, which is a multi-enzyme enzyme, is what catalyzes the fatty acid production process in the body. The fatty acid synthase (FAS) enzyme system is made up of two polypeptides with a molecular weight of 272 kilodaltonse [15],both of which are identical in every way apart from their level of activity. The various components of this system share a common substrate that is transported back and forth. This enzyme catalyzes the conversion 0f acetyl_CoA and mal0nyl-CoA into palmitat (C16:0, a long_chain saturated fatty acid) when NADPH[16]is present. The palmitate produced is a byproduct of this reaction.aims and purposes In individuals suffering from RA in Iraq, lipid profiles, fasting insulin levels, and IL-17A levels were investigated.

Materials and methods

Total DNA extraction

Following the manufacturers instructi0ns, gen0mic DNA was extracted from the participants' peripheral blood using the Favorgene® kit Gen0mic DNA Purificati0n Kite. Only sampls with adequate purity ratios (A260//A280 = 1.7 - 2) were used in the subsequeent studies. The DNA sample was stored at a cold -20 degrees Celsius until analysis could be performed. The ApaI (rs7975232) VDR SNP was identified using p0lymerase chaine reaction with restrictione fragment length polym0rphism (PCR_RFLP) using primer sequences (F: CAGAGCATGGACAGGGAGCAA and R:G AACTCCTCATGGCTGAGGTCTC).

Measurement of Serum Lipid Profile

Total cholesterole, triglyceride, high density 1ipoprotein, 1ow density lipopr0tein, and very 1ow density lip0protein were all calculated using spectrophotometric techniques. These procedures were carried out as per the manufacturer's guidelines.

Serum of fatty acid synthase FAS and IL-17A

Measurement

The Sandwich-ELISA was used to estimate FAS and IL-17A concentrations. The Human FAS-specific antibody has been pre_coated onto the micro EL1SA plate that is inclouded in the kite. Standards or samples are introduced into miicro EL1SA plate wells and then mixed with the proper antibody. After incubation, a bi0tinylated detection antib0dy is sequentially added to each microplate; this antibody recognizes and binds to the Avidin-Horseradish Peroxidase (HRP)-conjugated Human FAS and IL_17A. They purge the setup of any stray parts. The substrate solution is dispensed into the individual mold cavities. Wells containing human FAS and IL-17A, a biotinylated detection antibody, and an Avidin-HRP conjugate are required for the development of a blue color. The use of the stop solution terminates the enzyme-substrate reaction, explaining the transition from green to yellow. The spectrophotometer is used to measure the optical density (OD) at a wavelength of 450 nm 2 nm. There is a one-to-one relationship between the OD value and the concentration of human FAS in the sample.

Rresults

The VDR gene was amplified and digested by the restriction enzyme (ApaI) to reveal two different

alleles: the AA (C//C) allele, which has tw0 bands with a m0lecular size 0f 532 and 214base pairs, and the aa (A//A) alle1e, which has one band with a molecular size of 746 base pairs. The Aa (C//C) allele was also digested by ApaI (RE) as shown in figure (1).

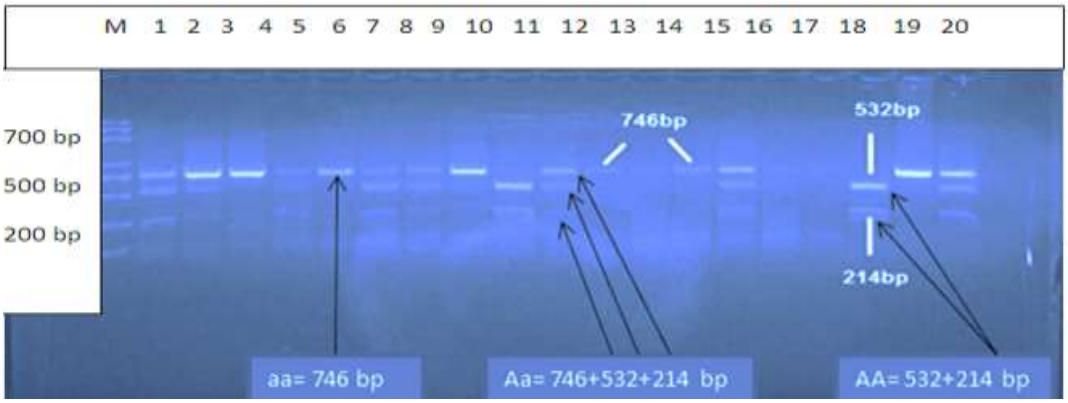

Figure (1): "Electrophoretic pattern represent VDR SNP rs7975232 SNP

The frequency of the VDR_ SNP (C>A rs7975232) & its association together with age and body mass index are summarized as shown in Table (1).

Table (1): The relationship between VDR: SNP C>A rs7975232 in RA gr0up with Age and BMI

PARAMETERS		Genotypes/ RA n =45 (%)			
		AA 19(42%)	Aa 11(24%)	aa 15(34%)	P-value
Age	<50 20(55%)	10(22)	7(16)	5(11)	
	≥50 26(45%)	9(20)	4(9)	10(20)	0.032*
BMI	<25 29(64%)	11 (24)	6 (13)	8 (18)	0.082
	≥25 16 (36%)	8 (18)	5 (11)	7 (16)	

Fragmentation of amplicons of the VDR SNP (C>A rs7975232) gene classifies each participant into one of three groups: those who are homozygous for the A allele (AA), who are heterozygous for the allele (Aa), or who are homozygous for the allele (aa). Table 2 displays these findings. For the VDR(ApaI SNP (rs7975232), the frequencies of the normal genotype (AA) were 20% (n = 9), the heterozygous genotype (Aa) was 22% (n = 10), and the homozygous genotype (aa) was 58% (n = 26) among the CONT patients. Three-and-a-half percent of people had the allele with the letter a, whereas sixty-one percent had the allele with the letter A. 42% (n = 19) of the RA group had the normal genotype AA, 31% (n = 14) had the heterozygous genotype Aa, and 27% (n = 12) had the homozygous genotype aa. The A allele was the most common, accounting for 59% of the population, while the an allele was the most common, at 41% ,as shown in table (2).

Table (2): Genotyping of VDR SNP (C>A rs7975232) and allele frequency

Genotypes	CONT n=45 (%)	RA n= 45(%)	Total	OR (CI 95%)	p-value
AA	9 (20)	19 (42)	28	1.0 (Reference)	0.000*
Aa	10 (22)	14 (31)	24	2.88 (1. 34-4.54)	
aa	26 (58)	12 (27)	38		
Total	45	45	90		
HWE(Alleles frequency) (p+q=1)					
A	39%	59%	2.12 (1.21-5.34)		0. 000*
a	61%	41%			

This study found that the genotyping frequencies of the VDR SNP (C>A rs7975232) differed significantly (p 0.05) between the RA group and the CONT group. The results suggest that possessing the VDR ApaI alleles A or an is associated with an increased risk of OST in comparison to women who have the Aa genotype. According to Hardy-Weinberg equilibrium (HWE), the frequency at which the ApaI SNP for VDR is genotyped is shown in the figure. Both the OST and control groups contributed to this data collection.

The current study describes the demographics of the 90 people who took part in it; 45 of them had RA, while the other 45 acted as controls.

According to Table 3, the overall prevalence of RA is 32% in males whereas it is 68% in females.The effects of the VDR SNP (C>A rs7975232) in AA, Aa, and aa genotypes of RA and CONT groups were analyzed by comparing the mean and standard deviation of FAS and IL-17A in these groups. Tables (4) and (5) show the findings of these contrasts. Figure 2 shows that 68% of patients have positive RF, while 32% of patients with a P-value less than 0.05 have negative RF. There are two subsets of the control group: those with positive RF, who account for around 10% of the total, and those with negative RF, who make up the remaining 90%. Table 6 shows that while there was statistical significance for a rise in cholesterol and LDL in the study group (p0.001 and p0.001, respectively), there was also statistical significance for a decrease in HDL (p0.001) and no significance for increases in triglycerides or very low density lipoprotein (p0.1 and p0.09) as shown in figure (3), (4), (5) and (6).

Table (3): Distribution of Gender among RA and CONT groups

Sex	Study groups		P-value
	RA patients	Control	

Female	31	23	0.677 NS
%	68%	51%	
Male	14	22	
%	32%	49%	
Total	45	45	

Table (4): Comparis0n of leveles of FAS (ng//m1) between a1l gen0types of study groups

Genotype (Co-dominant)	(FAS ng/ml) levels in C0NT (Meane ± SD)	(FAS ng//ml) 1evels in RA (Mean ± SD)	p_ value
AA.	0.9±0.009	1.4±0.9	0.000
Aa	1.26±0.004	1.69±0.8	0.000
aa	1.05±0.005	1.91±0.4	0.000
AA vs. Aa+ aa (Dominant)	1.04±0.001	1.87±0. 5	0.000
AA+ Aa vs. aa (Recessive)	0.78±0.008	1.55±0.7	0.000
AA+ aa vs. Aa (over dominant)	0.69±0.002	1.77±0.7	0.000

Table (5): Comparis0n of 1evels of IL-17A (pg/ml) between a1l gen0types of study gr0ups

Genotype (Co-dominant)	(IL-17A pg/ml) 1evels in C0NT (Mean±SD)	(IL-17A pg/ml) levels in RA (Mean± SD)	p_value
AA	105.3±11.2	188.8±12.7	0.000
Aa	101.6±12.2	199.8±12.9	0.000
aa	100.5±12.1	206.7±12.1	0.000
AA vs. Aa+ aa (Dominant)	99. 4±9.1	196.87±12.2	0.000
AA+ Aa vs. aa (Recessive)	107. 8±12.2	198.4±12.3	0.000
AA+ aa vs. Aa (over dominant)	105.9±12.1	206.9±10.5	0.000

Table (6): TC, TG, VLDL, LDL, and HDL level

PARAMETER	RA patients		Control		P-value
	Mean	SD	Mean	SD	

TC (mg/dl)	169.14	34.21	149.22	21.57	<0.05*
TG (mg/dl)	149.33	34.26	150.11	27.11	0.212 NS
HDL (mg/dl)	35.55	6.33	44.09	8.33	<0.05*
LDL (mg/dl)	109.08	30.02	71.09	26.04	<0.01*
VLDL (mg/dl)	29.21	7.09	31.09	4.99	0.054NS

Figures (2) and (3) displays the resu1ts 0f the current study which conclude that the 1evels of RF, FAS and 1L_17A in the patient group are significantly higher than the levels in the contr0l group, with a p_value (0.001) indicating a statistically significant difference.

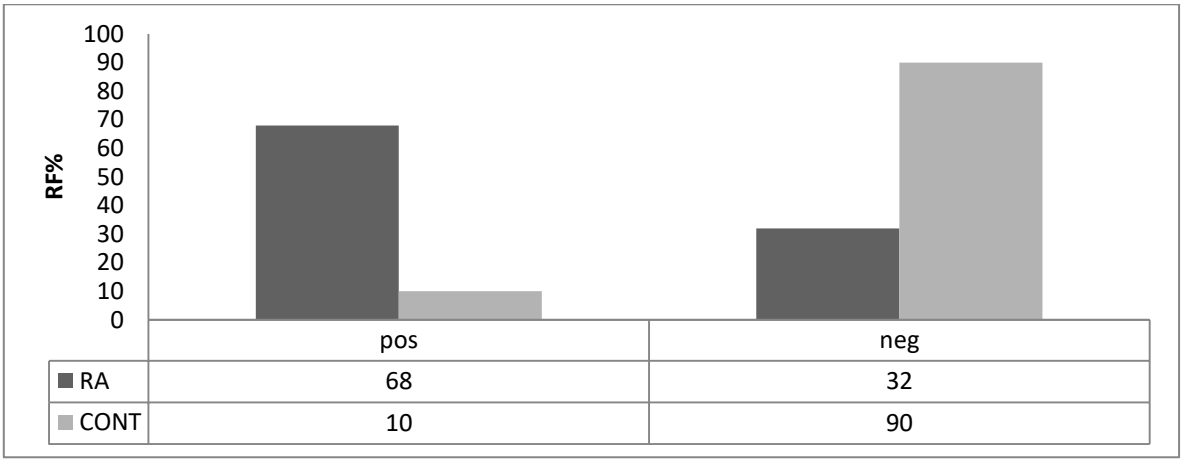

Figure (2): Percentage of RF in RA and CONT groups

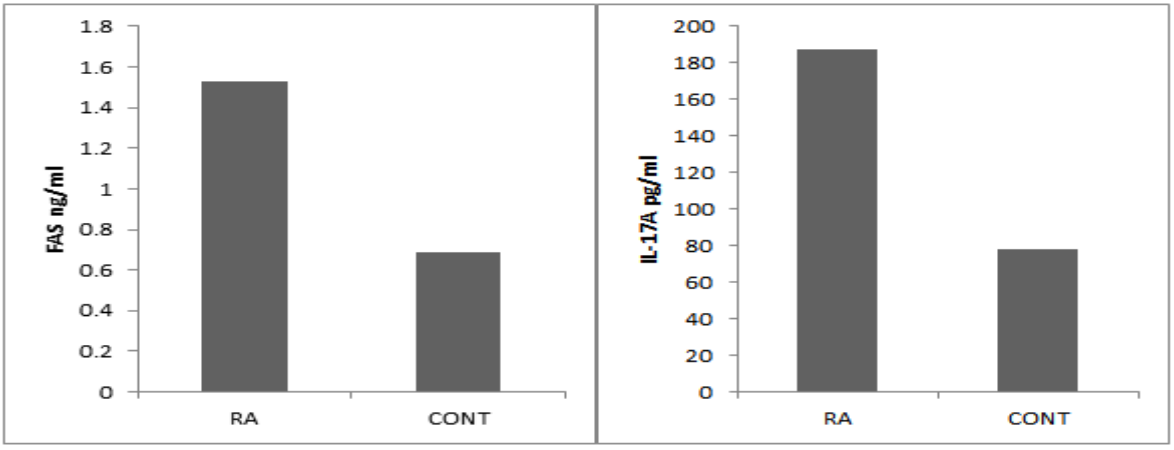

Figure (3): FAS (ng/ml) and IL-17A (pg/ml) levels in the RA group and CONT group

Discussion

The disease's prognosis can be projected based on the presence of specific clinical and laboratory evidences[17],and early treatment of rheumatoid arthritis is now achievable as a result of improvements made to the criteria used to classify the condition. In the absence of appropriate treatment, the inflammatory illness known as

rheumatoid arthritis can lead to the destruction of the joints. Between the onset of symptoms and the diagnosis of a particular rheumatic disease, such as rheumatoid arthritis [18]a person who has inflammatory arthritis may go through various stages. The purpose of this study was to investigate whether or not FAS and IL-17A may be utilized as a predictive indicator in the process of making a diagnosis of RA. The purpose of this study was to assess the influence that the VDR SNP (C>A rs7975232) has on the RA and CONT groups by analyzing the mean standard deviations of FAS and IL-17A in persons who had the AA genotype, the Aa genotype, or both. According to the findings of the research, "there is a signficant diference in the 1evel 0f the FAS and IL_ 17A between the patients (1.53 ±o.48) and control group(0.69± 0.13), p-value (0.001), which meant that the patients gr0up had a greater level of both FAS and IL-17A than the control group had. Due to the beneficial effect that unsaturated fatty acids have on the treatment outcomes of rheumatoid arthritis patients, the use of a diet that is abundant in long-chain unsaturated fatty acids, in addition to the administration of medicine, ought to be the standard of care for RA patients. According to the findings of Taski et al. [19], eicosanoids that possess anti-inflammatory properties can be partially generated from fatty acids. Patients with rheumatoid arthritis who followed an anti-inflammatory diet that was high in fish oil had much reduced morning stiffness and fewer cases of sensitive, swollen joints, according to a number of studie[20-24]Galarraga et al. [25]discovered that out of 49 patients, 19 (39%) in the group that received cod liver oil lowered their daily NSAID doses by more than 30%, whereas just 5 (10%) in the group that received a placebo did so. According to the findings that were presented earlier, there were n0 discernible diffrences between the gr0ups in terms of the clinical measurements described earlier that were used to evaluate the disease activity of RA or the detected side effects. However, HDL levels decreased significantly (p-values of less than 0.001 for both variables); neither triglyceride nor very low density lipoprotein levels changed significantly (p-values of less than 0.10 and 0.09, respectively). The findings of this study showed that both cholesterol and LDL levels increased significantly in the study group (p-values of less than 0.001 for both variables). Both the concentration of the FFA mixture and the ratio of unsaturated to saturated fatty acids were found to have a significant impact on lymphocyte proliferation in vitro (p0.0001) [26-28].This was demonstrated through the use of fluorescent microscopy. According to the findings of the previous investigations, T-lymphocyte proliferation was inhibited in the laboratory when patients with rheumatoid arthritis (RA) abstained from food for a period of seven days. As Robert and Miossec [29].IL-17 is a one-of-a-kind tool and concept that is necessary to identify individuals who possibly benefit from these IL-17 targeted medications in RA. This discovery was made possible by the fact that IL-17 is a target for certain therapies. In addition, the development of predictive biomarkers of response has been kicked off by the appearance of several bioassays. In conclusion, it would appear that testing for FAS and IL-17 can be helpful in the diagnosis of RA as well as the ongoing monitoring of people who have RA.

Conclusions

There is now evidence that the vitamin D receptor SNP (C>A rs7975232) is a risk factor for the development of rheumatoid arthritis (RA), and this evidence has led to the recommendation of a new strategy for the diagnosis and follow-up of patients with RA.

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