Lower frequency of anti-citrullinated protein antibodies among early arthritis patients with high body mass index

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Abstract

Objective

To investigate the role of body mass index (BMI) in the phenotypic and genotypic characteristics of early arthritis patients.

Methods

We analysed the clinical and laboratory parameters from the baseline visit of patients (670 patients [78.51% women]) included in the PEARL study. The WHO definition for low weight, normal weight, overweight and obesity (BMI <18.5, 18.5–25, 25–30 or ≥30 kg/m², respectively) was applied. Anticitrullinated protein antibodies (ACPA) were studied by ELISA and HLA-DRB1 were genotyped by sequence specific oligonucleotide probes. The relationship between BMI classification and other variables was analysed using Kruskall-Wallis, Anova and Chi-Square tests. Then multivariate logistic regression was performed to establish the role of BMI in ACPA positivity and ordered logistic regression to establish its relationship with ACPA level.*

Results

Among the patients studied, 255 (38.06%) were considered overweight and 136 (20.3%) obese. High BMI patients had significantly more pain perception and disability than normal weight patients, whereas no clear differences in disease activity were observed between high BMI and normal weight patients. ACPA positivity was significantly less frequent in overweight and obese patients compared to normal BMI patients. This information was confirmed by adjusting for smoking habit and the presence of shared epitope.

Conclusion

Our data support the theory that high BMI patients suffer more frequently from ACPA-negative RA. Nevertheless, although no disease activity differences were observed, these patients showed higher pain and disability scores since the beginning of disease.

Key words

rheumatoid arthritis, early arthritis, anti-cyclic citrullinated peptide antibodies, body mass index

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Introduction

Obesity is becoming a pandemic condition in developed countries leading to an increased burden of associated diseases. The role played by body mass index (BMI) in rheumatoid arthritis (RA) is controversial. During the last few years, some studies have shown an increased risk of RA development among people with high BMI and obesity (1-6). In addition, patients with RA and higher BMI show higher levels of disease activity (DA), more pain and higher scores on disability scales (7-10). Lower remission rates (7-10) and worse response to treatment (11) have also been described in these patients. On the other hand, radiographic progression seems to be lower in overweight and obese RA patients (8, 12-15).

The controversy refers to the fact that higher levels of DA and worse response to treatment do not fit well with lower radiographic progression. Some studies suggest that this is due to different aetiopathogenesis of RA, being less frequent anti-citrullinated protein antibodies (ACPA) positive disease in obese patients (16-18). However, other studies do not support this hypothesis (13). In psoriatic arthritis patients and healthy volunteers, obesity is associated with higher MRI scores, although less synovitis and bone oedema are observed in obese RA patients (12, 13). There are few studies addressing the role of BMI in early arthritis patients. Therefore, the main purpose of this work is to deepen into the role of BMI in the phenotypic and genotypic characteristics of early arthritis patients.

Patients and methods

Design and patients

This is a cross sectional analysis of the baseline data from patients included in the PEARL (Princesa Early Arthritis Register Longitudinal) study, which includes patients who are referred to the Early Arthritis Clinic at Hospital Universitario La Princesa in Madrid (Spain) because of suspicion of arthritis.

The PEARL study started in 2001 and it is still ongoing, but the last patient included in the present analysis underwent the 24-month follow-up visit in September 2018.

The register protocol comprises the prospective longitudinal collection of socio-demographic, disease-related (clinical, laboratory and radiological variables) and treatment data in five protocolised visits (baseline, 6, 12, 24 and 60 months). Biological samples are systematically collected by protocol at each visit. There is no pre-established treatment protocol and all the therapeutic decisions rely on each responsible physician from the Rheumatology Department along the follow-up. The register evaluation visits were performed by the same two rheumatologists (AMO, IGA) in order to get a more accurate clinical evaluation, especially regarding joint counts. More detailed descriptions of the PEARL study have been previously published (19, 20).

To be included in this work patients had to meet the following criteria:

a) at least one swollen joint at baseline visit for less than one year;

b) availability of DNA sample for HLADRB1 genotyping; and

c) diagnosis of RA (ACR/EULAR 2010 RA criteria) (21) or undifferentiated arthritis (UA) (22) after excluding other aetiologies (microcrystalline arthritis, septic or viral arthritis, osteoarthritis, spondyloarthritis, or connective tissue diseases) at baseline or at the 24-month follow-up visit.

The PEARL study is conducted according to the principles expressed in the Helsinki Declaration of 1983 and it was approved by the Research Ethics Committee of Hospital Universitario La Princesa (PI-518). All patients signed a written consent at study entry. Samples and data from patients included in this study were provided by the Biobank Biobanco Hospital Universitario La Princesa (ISCIII B.0000763) and they were processed following standard procedures with the appropriate approval of the ethics and scientific committees.

Variables

We used the WHO definition for low weight, normal weight, overweight and obesity (BMI <18.5, 18.5–25, 25–30 or ≥30 kg/m^2, respectively).

All clinical and laboratory data used in this study were collected at baseline visit, allowing us to calculate DAS28

Table I. Descriptive variables (sociodemographic, disease related and laboratory data) of the PEARL registry population related to the BMI.

BMI (kg/m^2)	Low weight (<18.5)	Normal weight [18.5-25)	Overweight [25-30)	Obese (≥30)	Total	*p*-value
n (%)	13 (1.94)	266 (39.7)	255 (38.06)	136 (20.3)	670 (100)	-
Age at onset (years) median [IQR]	52.3 [24.5-61.1]	49.1 [38–63.2]	58.2 [47.4-68.9]	57.7 [48.3-69.7]	54.4 [42.8-66.8]	**<0.01**
Sex (female/male) (%)	100/0	86.09/13.91	72.55/27.45	72.79/27.21	78.51/21.49	**<0.01**
Smoking habit n (%)						
Non smoker	4 (33.33)	126 (48.65)	131 (54.13)	78 (58.65)	339 (52.48)	0.134
Ever smoker	8 (66.67)	133 (51.35)	111 (45.87)	55 (41.35)	307 (47.52)	
Time to disease (mos.) median [IQR]	2.7 [1.7 – 4.03]	4.93 [2.73 – 8.2]	4.6 [2.76 – 7.83]	4.7 [2.33 – 8.8]	4.7 [2.63 – 8.13]	0.058
DAS28 mean (SD)	4.85 (1.67)	4.15 (1.48)	4.26 (1.45)	4.58 (1.61)	4.29 (1.51)	0.579
HUPI median [IQR]	7.5 [6 - 10]	6.5 [3.5 - 9]	6.5 [4.5 - 9]	7.5 [5.5 - 10.5]	7 [4 – 9.5]	**0.013**
HAQ median [IQR]	0.875 [0.75-1.25]	0.875 [0.25-1.375]	0.875 [0.375-1.5]	1.125 [0.625-1.75]	0.875 [0.37-1.625]	**<0.01**
VAS Pain (mm) median [IQR]	34 [16-53]	39 [16-60]	46 [20-61]	50 [35-65]	44.5 [21-62]	**<0.01**
RF + n (%)	2 (15.38)	130 (48.87)	118 (46.27)	64 (47.06)	314 (46.87)	0.125
ACPA + n (%)	5 (38.46)	124 (48.44)	89 (36.18)	46 (35.66)	264 (40.99)	**0.021**
Shared epitope (%)						
No copies	72.73	39.82	50	47.37	45.88	0.131
1 copy	18.18	46.15	41.04	43.86	43.19	
2 copies	9.09	14.03	8.96	8.77	10.93	
CRP (mg/dl) median [IQR]	0.4 [0.13-2.83]	0.5 [0.1-1.29]	0.6 [0.3-1.7]	0.7 [0.3–2.08]	0.6 [0.2-1.5]	**<0.01**
2010 ACR-EULAR criteria n (%)	6 (46.15)	153 (57.52)	144 (56.47)	81 (59.56)	384 (57.31)	0.796

n: number; IQR: interquartile range; mos: months; DAS28: Disease Activity Score based on 28 joints count; SD: standard deviation; HUPI: Hospital Universitario Princesa Index. HAQ: Health Assesment Questionnaire; VAS: Visual Analogue Scale; RF +: rheumatoid factor positive; ACPA+: anti-citrullinated peptide antibodies positive; CRP: C-reactive protein; cr: criteria.

(23) and HUPI (24), in order to assess disease activity. Disability was determined using the Health Assessment Questionnaire (HAQ) (25).

ACPA were measured using a second-generation anti-citrullinated cyclic peptide enzyme immunoassay (EIA; Euro-Diagnostica Immunoscan RA; positive >50 U/ml) until October 2010 and then using a third-generation EIA (QUANTA Lite CCP2 IgG and IgA, Inova Diagnostics; positive >40 U/ml). Therefore, in order to unify the data, ACPA levels were classified as negative if below the manufacturer's limit, low if above this limit but below the median of the positive population and high when above the median of the positive population.

HLA-DRB1* medium resolution typing was carried out using polymerase chain reaction (PCR) and sequence specific oligonucleotide probes (SSOP) Luminex method with LABType SSO (One Lambda Inc., Canoga Park, CA), according to the manufacturer's instructions. Briefly, target DNAs were PCR-amplified using HLA-DRB1* group-specific primers. The biotinylated PCR products were denatured and hybridised with specific probes bound to colored coded microspheres. Phycoerythrin conjugated Streptoavidin was used to label and reveal reactions and a flowanalyzer, LABScan3D, was used to identify fluorescent intensity on each microsphere. The software HLA Fusion 4.1 (One Lambda Inc) was used to assign the HLA-DRB1* typing.

Statistical analysis

The statistical analysis was carried out using Stata 12.1. The descriptive analysis was performed by calculating the median and the interquartile range (IQR) for the variables that did not follow a normal distribution. The mean and standard deviation (SD) were calculated for those variables with a normal distribution. Kruskall-Wallis test was used to compare quantitative variables that did not follow a normal distribution and BMI classification. For those variables following a normal distribution, Anova with Bonferroni correction was used. Chi-Square test was used to compare qualitative values and BMI classification. Fisher's exact test was used when the number of patients in each one of the studied variables subgroups was less than 5. *p*-values less than 0.05 were regarded as statistically significant. Shapiro-Wilk test was used to assess the normality of the different populations.

To evaluate the impact of BMI on the presence of ACPA, several multivariate analyses were performed. First, a multivariate logistic regression (*logit* command of Stata) was fitted including ACPA presence (positive/negative) as the dichotomic dependent variable and, as independent variables, smoking habit in interaction with the number of shared epitope alleles, as well as BMI, either as a categorical variable (model 1) or as a continuous variable (model 2). On the other hand, to determine whether BMI affected ACPA level, we fitted a multivariate ordered logistic regression (*ologit* command of Stata) in which the dependent variable ACPA was clustered in negative, low or high levels, and the independent variables were the same as the ones described previously for the logistic regression.

Results

Patients

A total of 670 patients of the PEARL study were included for this analysis. As shown in Table I, 526 (78.51%) were women, 384 (57.31%) fulfilled 2010 ACR-EULAR RA classification criteria and the remaining patients were considered UA (Supplementary Table S1 provides additional information depending on diagnosis). More than 50% patients were classified as overweight and obese (Table I). These patients

were significantly older at disease onset compared to normal weight patients and the proportion of men was significantly higher in overweight and obese patients than in the normal and low weight ones (Table I). In addition, patients with a higher BMI had significantly lower education level, were married in a greater proportion and more frequently treated with statins (Suppl. Table S2).

Clinical presentation at baseline depending on BMI

Obese patients showed significantly higher HAQ score and pain perception (Table I, Fig. 1) than normal weight patients. However, no significant differences were observed in DA when it was calculated with DAS28, while low weight and obese patients showed slightly and significantly higher DA when it was calculated with HUPI (Table I). In this regard, all these variables showed a U-shaped distribution with higher values in both extremes of BMI (Fig. 1).

Considering the individual variables included in both DA indexes, obese patients showed significantly higher tender joint count (TJC) and patient global assesment (PGA), whereas neither swollen joint count (SJC) nor erythrosedimentation rate (ESR) were significantly different between BMI groups (Suppl. Table S2, Fig. 2). On the other hand, C-reactive protein (CRP) values were significantly increased in patients with higher BMI (Table I).

Effect of BMI on level and positivity of ACPA

In our population, ACPA positivity was significantly more frequent in patients with normal BMI than in the other groups (Table I). By contrast, BMI did not seem to have a significant influence on ACPA level (Suppl. Table S2, Fig. 3A). It has been described that ACPA positivity is clearly related to carrying shared epitope (SE) copies and being ever smoker. We did not find significant differences in these variables between BMI groups, although a trend to lower frequency of carrying two SE alleles in overweight and obese patients (8.96% and 8.77% respectively) compared to normal weight patients (14.03%) was observed (Table I, Fig. 3B)

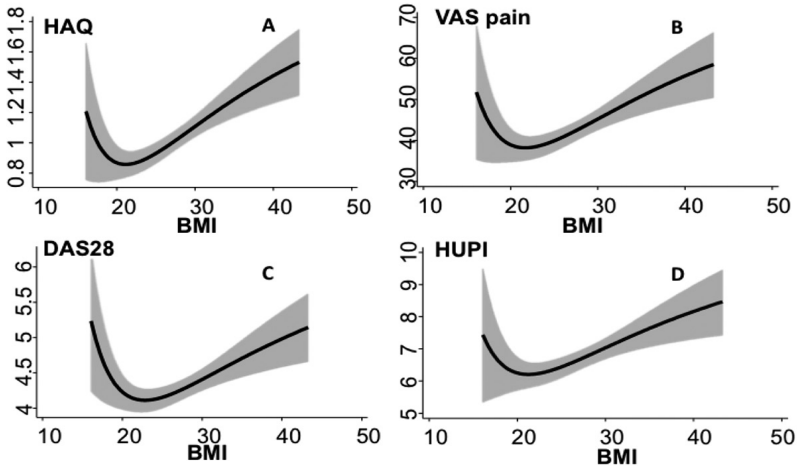

Fig. 1. Relationship between clinical severity parameters and body mass index in early arthritis patients. Relationship between BMI and disability assessed by HAQ (**A**), pain perception (**B**) and disease activity estimated either by DAS28 (**C**) or HUPI (**D**). Data are shown as the prediction for each outcome variable from estimation of a fractional polynomial prediction of BMI using the twoway fpfitci command of Stata 12.1. The 95% confidence interval is shown as a grey shadow.

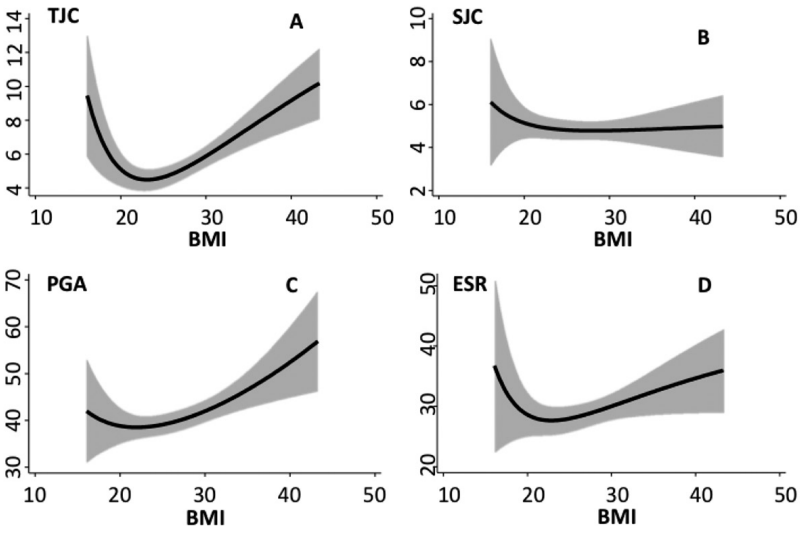

Fig. 2. Relationship between body mass index (BMI) and the different components of DAS28. Relationship between BMI and the individual variables included in DAS28 index: tender (**A**) and swollen joints (**B**), patient global assesment (PGA) (**C**) and erythrosedimentation rate (ESR) (**D**). Data are shown as the prediction for each outcome variable from estimation of a fractional polynomial prediction of BMI using the twoway fpfitci command of Stata 12.1. The 95% confidence interval is shown as a grey shadow.

Therefore, we analysed the effect of BMI adjusting for number of SE copies and smoker status. Obese patients showed an almost significant 40% lower possibility of being ACPA-positive (Table II, Model 1). This variable reached statistical significance when BMI was used as continuous variable (Table II, Model 2). Regarding ACPA level, there was a trend to lower ACPA level with increasing BMI, although statistical significance was not reached either with the categorical or with the continuous BMI variable (Suppl. Table S3).

Discussion

The main findings of this study are that early arthritis patients with higher BMI are more frequently ACPA-negative and show more pain and disability than those with normal weight, despite disease activity is not clearly higher in these patients than in normal weight patients. These findings are of great value because they have been obtained in early arthritis patients, avoiding the bias that long lasting RA studies can introduce because of the possibility of weight gain in these patients due to lower physical

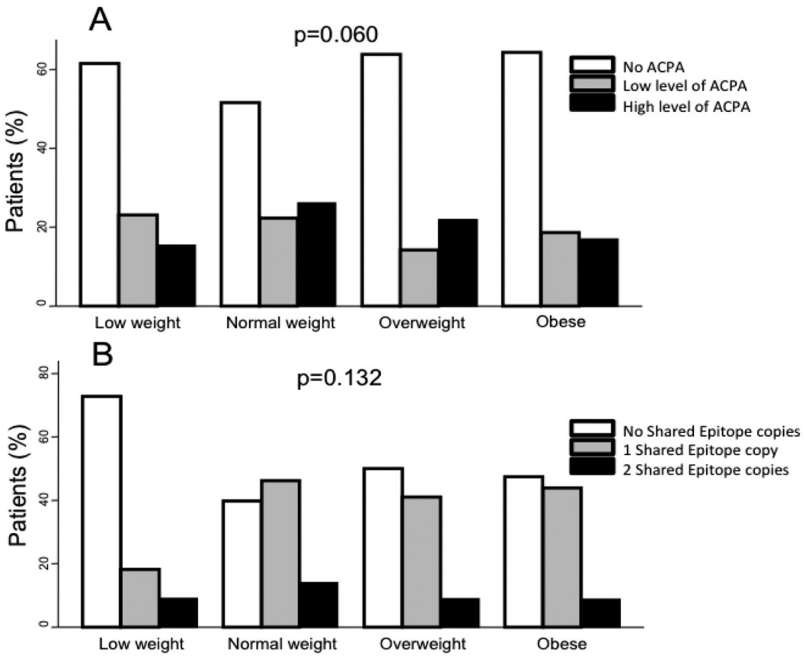

Fig. 3. Anti-citrullinated protein antibodies (ACPA) level (**A**) and shared epitope distribution (**B**) according to different categories of body mass index (BMI). Statistical significance was determined through Fisher's exact test and set to $p<0.05$.

Table II. Multivariate analysis of variables associated with ACPA positivity in patients with early arthritis.

ACPA positivity			
MODEL 1 - BMI as a categorical variable		OR [CI 95%]	*p*-value
BMI	Normal weight	Ref.	-
	Low weight	0.79 [0.207 – 3.011]	0.73
	Overweight	0.69 [0.466 – 1.047]	0.083
	Obesity	0.63 [0.389 – 1.031]	0.066
Smoking habit & SE copies	Non smoker - 0 copies	Ref.	-
	Non smoker - 1 copy	1.55 [0.928 – 2.607]	0.093
	Non smoker - 2 copies	4.11 [1.766 – 9.589]	**0.001**
	Ever smoker - 0 copies	1.073 [0.621 – 1.852]	0.799
	Ever smoker - 1 copy	2.73 [1.596 – 4.688]	**<0.001**
	Ever smoker - 2 copies	6.54 [2.43 – 17.566]	**<0.001**
MODEL 2 - BMI as a continuous variable			
BMI		0.96 [0.92 – 0.99]	**0.044**
Smoking habit & SE copies	Non smoker - 0 copies	Ref.	-
	Non smoker - 1 copy	1.55 [0.92 – 2.6]	0.093
	Non smoker - 2 copies	4.18 [1.79 – 9.72]	**0.001**
	Ever smoker - 0 copies	1.04 [0.61 – 1.8]	0.865
	Ever smoker - 1 copy	2.73 [1.59 – 4.68]	**<0.001**
	Ever smoker - 2 copies	6.7 [2.5 – 17.96]	**<0.001**

ACPA: anti-citrulinated peptide antibodies; BMI: body mass index; OR: odds ratio; CI: confidence interval; SE: shared epitope.

activity secondary to higher disability. Our data on more pain perception and more disability on HAQ scale in early arthritis patients with high BMI (overweight and obese patients) are in accordance with those previously described in a systematic review and meta-analysis by Vidal *et al*. in 2015 (8). We expected, as these authors described, that this could be due to increased disease activity in obese patients compared to normal weight patients. However, our results do not support differences in disease activity calculated with DAS28.

In this regard, studying the individual components of DAS28 we found that PGA and TJC were higher in overweight and obese patients, but not ESR and SJC. A slight but significantly higher disease activity was detected in patients with higher BMI when activity was calculated with HUPI, an index that adjusts TJC and ESR for gender. This can be explained by the higher proportion of male patients in the overweight and obese groups of our population. We cannot ascertain whether the SJC is similar in all groups because detecting clinical synovitis is a challenge in obese patients and ultrasound joint counts are not performed by protocol in PEARL. In accordance to this there are some studies like the one performed by Goosens *et al*. (26) that suggest that in high BMI RA patients both SJC and DAS28 seem to be undervalued by clinical assessment when compared to US. However, there is also disparity in the comparison of acute phase reactants between obese patients and normal weight patients. Our results show that obese patients do not have increased levels of ESR. Nevertheless, these patients present significantly higher levels of CRP with age in men (27), but not in women. There are also studies that show the relationship between a higher BMI and a higher CRP (28, 29), which is also observed in this study.

On the other hand, the role played by BMI in the development of ACPA-positive RA is controversial (13, 16-18), and our results clearly show that being overweight and obese leads to ACPA-negative RA more frequently than in normal weight cases. Although overweight and obese patients showed non significant lower frequencies of being ever smoker or carrying SE copies, even adjusting for this confounders, the effect of high BMI was associated with a 40% lower frequency of developing ACPA-positive disease. As some authors have proposed before, RA in high BMI patients can develop from different aetiopathogenic mechanisms, involving adipocytokines like leptin and other proinflammatory cytokines from white adipose tissue (30).

This study has several limitations. First, it is a single-centre study and some biases may affect our population. In fact, obesity had been proposed to be a risk factor of ACPA-negative RA develop-

ment in young women (1), but we found higher proportions of men among overweight and obese patients than in normal or low weight patients from our population. Second, PEARL has not established by protocol ultrasound joint counts, so we cannot confirm whether obese patients show subclinical synovitis. And finally, although hands and feet x-ray are scheduled in PEARL, no relevant differences were observed (data not shown). This could be explained by our previous results describing that due to an increasing adherence to early disease modifying anti-rheumatic drugs (DMARD) prescription and treat-to-target (T2T) strategy over the last decade, almost no progression of the erosion component of the Sharp van der Heijde score is detected (20).

In conclusion, in our early arthritis population, overweight and obese patients have more pain and dysfunctionality and suffer predominantly an ACPA-negative disease. This could suggest different aetiopathogenic factors for arthritis development in these patients. These results should be validated in other populations and additional factors, such as the role of adipocytokines and genetic variants, should be investigated.

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