TRANSLATIONAL SCIENCE

Discovery of an autoantibody signature for the early diagnosis of knee osteoarthritis: data from the Osteoarthritis Initiative

María Camacho-Encina,[1] Vanesa Balboa-Barreiro,[2] Ignacio Rego-Perez,[3] Florencia Picchi,[1] Jennifer VanDuin,[4] Ji Qiu,[4] Manuel Fuentes,[5] Natividad Oreiro,[6] Joshua LaBaer,[4] Cristina Ruiz-Romero ![ORCID],[1] Francisco J Blanco ![ORCID] [7]

ABSTRACT

Objective To find autoantibodies (AAbs) in serum that could be useful to predict incidence of radiographic knee osteoarthritis (KOA).

Design A Nucleic-acid Programmable Protein Arrays (NAPPA) platform was used to screen AAbs against 2125 human proteins in sera at baseline from participants free of radiographic KOA belonging to the incidence and non-exposed subcohorts of the Osteoarthritis Initiative (OAI) who developed or not, radiographic KOA during a follow-up period of 96 months. NAPPA-ELISA were performed to analyse reactivity against methionine adenosyltransferase two beta (MAT2β) and verify the results in 327 participants from the same subcohorts. The association of MAT2β-AAb levels with KOA incidence was assessed by combining several robust biostatistics analysis (logistic regression, Receiver Operating Characteristic and Kaplan-Meier curves). The proposed prognostic model was replicated in samples from the progression subcohort of the OAI.

Results In the screening phase, six AAbs were found significantly different at baseline in samples from incident compared with non-incident participants. In the verification phase, high levels of MAT2β-AAb were significantly associated with the future incidence of KOA and with an earlier development of the disease. The incorporation of this AAb in a clinical model for the prognosis of incident radiographic KOA significantly improved the identification/classification of patients who will develop the disorder. The usefulness of the model to predict radiographic KOA was confirmed on a different OAI subcohort.

Conclusions The measurement of AAbs against MAT2β in serum might be highly useful to improve the prediction of OA development, and also to estimate the time to incidence.

Key messages

What is already known about this subject?
► Autoantibodies (AAbs) are used as biomarkers in autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus. In these and other plethora of disorders, they can be detected at asymptomatic stages.
► Although the presence of AAbs has been reported in the serum of patients with osteoarthritis (OA), they had not been previously associated with the incidence or progression of this disease.

What does this study add?
► A specific panel of AAbs has been detected at baseline in individuals developing incident radiographic knee OA (KOA) during a 96-month follow-up period, compared with those who remained healthy.
► Reactivity levels of AAbs against the beta subunit of the methionine adenosyltransferase (MAT2β-AAb) II enzyme are positively correlated with the time to OA incidence.

How might this impact on clinical practice or future developments?
► The addition of MAT2β-AAb to a prognostic clinical model of incident radiographic KOA might significantly improve the identification at baseline of those individuals who will develop the disorder during a follow-up period of 96 months.

INTRODUCTION

Osteoarthritis (OA) is the most common arthritic disease involving movable joints and it is increasingly important in current ageing populations, leading to patient chronic disability.[1 2] The current diagnostic methods are insensitive to detect the small changes occurring at early stages, when OA is characterised as an asymptomatic disease.[1] To solve this problem, a molecular level of interrogation is hypothesised as the only alternative to detect the earliest phases of the disease process.[2]

Although OA is not considered an autoimmune disease, cell stress and extracellular matrix degradation may activate maladaptive repair responses, including pro-inflammatory pathways of innate immunity.[3] Activation of the immune response usually involves the production of immunoglobulins against self-proteins or autoantibodies (AAbs), which can be detected in sera and used as biomarkers for early diagnosis.[4 5] In this field, the Nucleic-Acid Programmable Protein Array (NAPPA) strategy has been widely used to detect AAbs in a high-throughput manner in many diseases,[6 7] and has been employed in an exploratory study on sera from patients with OA.[8] The NAPPA arrays are

generated by printing full-length cDNAs encoding the target proteins with a tag on the surface of the array.[9] Proteins are then transcribed and translated by a mammalian cell-free system and captured in situ by immobilised antibodies specific for the tag encoded at the carboxy-terminus of the amino acid sequence.[10]

The Osteoarthritis Initiative (OAI) is an ideal target population to detect relevant biomarker characteristics of earlier stages of the disease. It is a multi-centre, longitudinal and observational cohort study that has enrolled 4796 individuals which have been followed during 96 months.[11 12] Among all these subjects, the OAI comprises participants without clinically significant knee osteoarthritis (KOA) at baseline, but selected on the basis of having specific characteristics that give them an increased risk of developing incident symptomatic KOA (incidence subcohort), and a reference control group whose participants did not have neither symptomatic KOA nor risk factors at baseline (non-exposed subcohort).

In the present study, serum samples at baseline from the incidence and non-exposed subcohorts of the OAI were analysed using NAPPA technology for the discovery of an AAb profile that could be associated with an early and asymptomatic stage of the disease. The objective was to detect AAbs useful to identify those asymptomatic individuals who will develop radiographic KOA before 96 months, and examine the putative relationship between their levels in serum and the time for OA incidence.

MATERIALS AND METHODS
### Definition of incident radiographic KOA
In this case–control study, two main outcomes group, with one study knee per subject (target knee), were defined: the incident (n=146) and the non-incident group (n=181), both without clinically relevant radiographic KOA at baseline (Kellgren and Lawrence (KL) grade=0–1) in at least one knee (target knee). Incident radiographic KOA was defined by KL grade ≥2 in the target knee at some point between 12 and 96 months of follow-up.

Study design
A two-stage discovery approach (screening and verification) was designed to analyse the presence and putative usefulness of KOA-associated AAbs to predict the incidence of the disorder. The sera were blindly analysed and belonged to Caucasian participants from the incidence and non-exposed subcohorts of the OAI at the baseline visit (181 non-incidents and 146 incidents at 96 months). The prognostic clinical model generated to predict KOA development was later replicated in a total of 108 participants (65 non-incidents and 43 incidents at 96 months) from the progression subcohort of the OAI at the baseline visit. Detailed information about the workflow of the study is summarised and illustrated in figure 1.

NAPPA profiling of serum AAbs
The NAPPA core Centre for Personalised Diagnostics (CPD) at the Biodesign Institute (Arizona State University, USA) had all human genes from the DNASU (www.dnasu.org) on six different array sets: HC1−HC6. The HC5 set was selected for the screening on the basis of having the greatest number of genes that could be related with OA pathogenesis according to bibliography (listed in online supplementary table S1). The quality of DNA printing, protein expression and detection of the NAPPA slides were performed using the standard procedure of CPD,[13] but incubating the expressed slides overnight with 150 μL of 1:20 (v/v) diluted serum. A titration assay was performed using
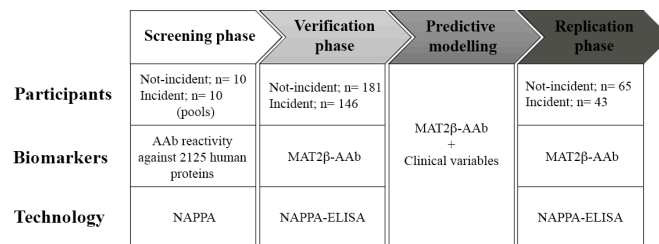
Figure 1 Study design. A two-stage discovery approach (screening and verification) was designed to investigate the putative utility of AAbs to predict OA development. Sera from the non-exposed and incidence OAI subcohorts at baseline without radiographic KOA (KL=0–1) in at least one knee (target knee) were analysed in these two phases. Incident radiographic KOA was defined by KL ≥2 at 12–96 months of follow-up. In the *screening phase*, reactivity levels of AAbs against 2125 proteins were evaluated in 10 pooled serum samples at baseline per Study Group (incident and non-incident) using the NAPPA platform. Each pool was prepared by mixing equal volumes of 10 individual sera. In the *verification phase*, the sensitivity and specificity of the baseline levels of MAT2β-AAb to predict KOA incidence was confirmed by applying the NAPPA-ELISA technique on a total of 327 individual sera at baseline, which included the 200 samples used at the screening. Then, a logistic regression model was developed combining different clinical variables and MAT2β-AAb levels. The clinical variables selected as covariates are listed in table 2. Finally, the proposed prognostic model was replicated in 108 individual sera at baseline from participants of the progression subcohort of the OAI without radiographic KOA. AAbs, autoantibodies; KOA, knee osteoarthritis; KL, Kellgren and Lawrence; MAT2β, methionine adenosyltransferase two beta; NAPPA, Nucleic-acid Programmable Protein Array; OA, osteoarthritis; OAI, Osteoarthritis Initiative.

serum dilutions from 1:20 down to 1:200 to identify an optimal dilution factor that provided an acceptable background without overwhelming the true signals.

The signal intensities obtained in the assay were normalised as described.[14] To determine positive AAb response, a cut-off level was calculated by median intensity absolute deviation rule from all the spots through all the serum pools. The mean and SD of the mean were obtained for the incident and non-incident groups. The antigens that did not exhibit intensities over the cut-off were eliminated. A differential spot analysis was performed with the remaining antigens by Wilcoxon Rank-Sum test (p value<0.05) and the area under the curve (AUC) at 95% specificity were calculated using the pROC package in R. In addition, AAb candidates were qualitatively examined by visual analysis through all the slides by adjusting in identical black and full colour threshold scale.

NAPPA-ELISA assay
In all, 500 ng of full-length methionine adenosyltransferase two beta (MAT2β) human recombinant protein fused to glutathione S-transferase (GST) were synthesised in vitro using the HeLa cell lysate-based protein in vitro transcription/translation (IVTT) system (Thermo Fisher Scientific). NAPPA-ELISA assay was performed as previously described[8] with some variations. 96-well plates coated with 40 ng of anti-GST antibody were blocked 4 hours at room temperature with 5% milk-1×phosphate-buffered saline with tween (PBST) (0.02% Tween20). 50 μL of the IVTT-expressed recombinant human protein was transferred to each well and incubated at 4°C overnight on shaker. Plates were then washed and incubated with 1:20 (v/v) diluted sera. The presence of specific MAT2β-AAb was detected by

incubation with horseradish peroxidase (HRP)-linked anti-Human IgG (Jackson ImmunoResearch Laboratories) diluted 1:1000 (v/v) in blocking buffer. After addition of tetramethyl-benzidine substrate, the absorbance signals at 450 nm were read on a Biotek Synergy four plate reader (Winooski, VT, USA). Levels of MAT2β-AAb were expressed in arbitrary units (a.u.) of absorbance.

Data analysis

The biological context network of MAT2β was analysed with the STRING (https://string-db.org/) bioinformatics webtool, using the K-means clustering method. Differences in the baseline reactivity levels of MAT2β-AAb were assessed by the Mann-Whitney U test and the association of this potential biomarker with OA incidence was evaluated with the OR. In addition, after assessment of cut-off values (tertiles) for MAT2β-AAb, patients with OA were categorised into high-level, medium-level and low-levels groups. Kaplan-Meier (KM) analyses were used to estimate and represent the survival probability, explained as the probability of not developing KOA in specific periods of time (12, 24, 36, 48, 72 and 96 months) depending on the tertiles of MAT2β-AAb reactivity levels of the participants.

To define prognostic models of OA, clinical data at baseline were obtained from the OAI database (https://data-archive.nimh.nih.gov/oai). Candidate non-radiographic clinical variables that may have prognostic value were selected based on the specific eligibility risk factor criteria for the incident subcohort of the OAI and prior published evidence suggesting a risk factor role in KOA incidence (online supplementary table S2). For all variables concerning the joint, knee-value predictors were recoded to indicate they were for the target knee. When neither or both knees have incident KOA, one of them was randomly selected and used in the analysis. In a primary step, univariable logistic regression analyses were employed to assess association between each variable with incident radiographic KOA. In a secondary step, a stepwise multivariable logistic regression analysis was performed to define a prognostic model of incident radiographic KOA.

The capacity of the models to predict OA incidence was evaluated using the AUC. The utility of the measurement of the reactivity levels of the potential biomarker was assessed by comparing the AUC of the covariates-only model with the AUC of the biomarker plus covariates model. Sensitivity, specificity and positive predictive value and negative predictive value were also estimated by the Youden Index to determine the validity and security of the models, and receiver operating characteristic (ROC) curves were evaluated. A nomogram was developed to facilitate application of the proposed prognostic model in a clinical setting. Finally, the validity of the proposed biomarker plus covariates model was evaluated by a replication analysis in a different set of participants from the OAI progression subcohort.

All regression analyses and KM curves were carried out using SPSS V.25 for Mac. Metrics were calculated using the pROC package in R.

Patient and public involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

RESULTS

Identification of AAbs associated with the incidence of KOA

To search for AAbs in the serum that could be associated with a future development of KOA, a comprehensive AAb profiling against 2125 full-length proteins was performed by NAPPA. It was carried out comparing pools of serum samples at baseline from two groups: the incident group, which contains participants belonging to the incidence subcohort of the OAI who did develop radiographic KOA during the 96-month follow-up (n=100, 10 pools), and the non-incident group, which contains participants from the non-exposed subcohort that remained radiographically healthy (n=100, 10 pools).

A signal cut-off >1.1 was employed to assure a sufficient margin between positive and negative AAbs reactivities. Among the 2125 screened proteins, a total of 1031 proteins showed positive immunoreactivity (online supplementary table S3). Mean and SD values for all the proteins expressed on the array for the incident and non-incident groups are summarised in online supplementary table S3. From all the proteins over the cut-off, the Wilcoxon Rank-Sum test identified a total of six AAbs that reacted with six different proteins on the array (online supplementary table S4). Furthermore, as shown in online supplementary figure S1, visually discernible differences demonstrated that the normalisation criteria employed did neither create signal differences that do not exist, nor destroy true signal differences.

### Verification of MAT2β-AAb levels as potential prognosis marker of OA incidence

To confirm the putative ability of any of these AAbs to predict the incidence of OA, anti-MAT2β was selected to be technically verified by NAPPA-ELISA in 327 individual serum samples, 200 of which (100 incidents and 100 non-incidents) were previously used at the screening phase. This selection was based on the role of MAT2β as negative regulatory subunit of the production of S-adenosylmethionine (SAMe), a main methyl donor in the human body and a widely used dietary supplement for OA management (AdoMet). In addition, its biological context network (online supplementary figure S2) suggests a bottleneck role of this protein in metabolic pathways that are known to be related with OA pathogenesis. After the subtraction of the negative control, we found positive reactivity against MAT2β in all the analysed sera. The higher baseline reactivity levels of MAT2β-AAb found in the incident group (0.58±0.22 vs 0.49±0.23 a.u., p=3.140E-04) verified our previous findings. The association of this potential biomarker with the clinical outcome and its ability to predict incidence of radiographic KOA were assessed and are summarised in table 1.

Furthermore, we detected a significant decrease in anti-MAT2β reactivity levels with the time to KOA onset (p value=0.002), as it is shown in online supplementary figure S3. The association between the baseline reactivity levels of MAT2β-AAb in sera and

Table 1 MAT2β-AAb model assessment in the verification phase

	Estimate	95% CI	
OR (p value)	5.99 (1.000E-03)	2.16	16.63
AUC	0.62	0.56	0.68
Sensitivity (%)	86	80	91
Specificity (%)	39	31	46
PPV (%)	53	50	57
NPV (%)	78	70	85

AUC, area under the curve; MAT2β-AAb, methionine adenosyltransferase two beta-autoantibody; NPV, negative predictive value; PPV, positive predictive value.
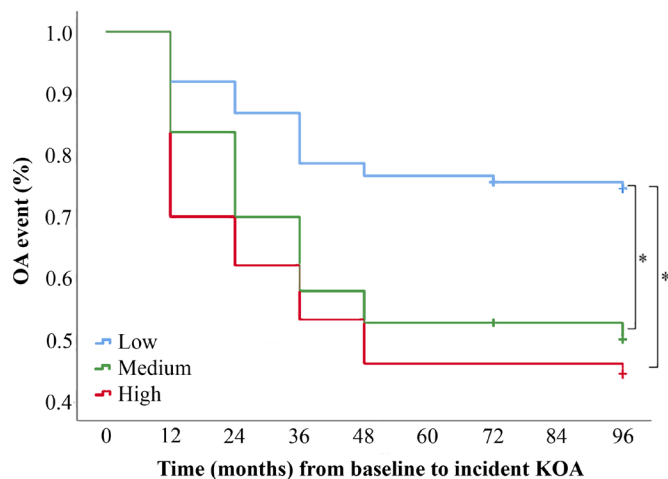
Figure 2 Association of biomarker levels with the time to KOA incidence. Kaplan-Meier reliability analysis for MAT2β-AAb in the OAI participants included in this work, classified into three groups (low level, medium level and high level) after the calculation of a cut-off value (MAT2β-AAb tertiles). *Level of significance below 0.05 by the Log-Rank test. KOA, knee osteoarthritis; MAT2β-AAb, methionine adenosyltransferase two beta-autontibody; OAI, Osteoarthritis Initiative.

the time to OA incidence were inferred by KM curves (figure 2). Individuals with low AAb levels at baseline (range=2.00E-3–0.39 a.u.) had a significant lower risk to develop KOA sooner in time than those with high (range=0.60–1.58 a.u., p=2.440E-04) or medium (range=0.39–0.60 a.u., p=5.000E-06) baseline levels. There were no significant differences in the time to KOA incidence when the high-level and medium-levels groups were compared (p=0.267).

Predictive modelling of KOA incidence with the combination of clinical variables and MAT2β-AAb levels

A unique covariates-only model including age, sex, body mass index (BMI), frequent knee bending activity, history of knee injury and the Western Ontario and McMaster Universities Osteoarthritis Index pain score was defined by stepwise multivariable logistic regression analysis. The clinical characteristics finally included in the model of the selected participants are presented in table 2. The results from the univariable logistic regression model of all the clinical variables analysed are shown in online supplementary table S5.

The clinical model defined herein yielded an AUC (95% CI) of 0.81 (0.76–0.86). The addition of MAT2β-AAb to this model significantly improved the capacity to predict radiographic KOA development in the target knee (p=0.048), yielding an AUC of 0.83 (0.78–0.87). Figure 3A,B shows the results from this regression analysis, together with the metrics and the ROC curves

obtained when comparing the covariates-only model with the MAT2β-AAb plus covariates model.

To facilitate the use of the proposed prognostic model in a clinical routine, a nomogram was developed (figure 3C) to determine the probability of a certain individual to develop KOA in the next 96 months.

Replication analysis in an independent OAI subcohort

The proposed MAT2β-AAb plus covariates model was replicated in an independent set of sera at baseline from participants without KOA belonging to the progression subcohort of the OAI. The clinical characteristics of this population are summarised in table 2. The AUC observed in this replication analysis was 0.76 (table 3), showing no significant differences with that obtained in the verification phase (p=0.218). In addition, the sensitivity, specificity and predictive values remained very similar in both verification and replication cohorts.
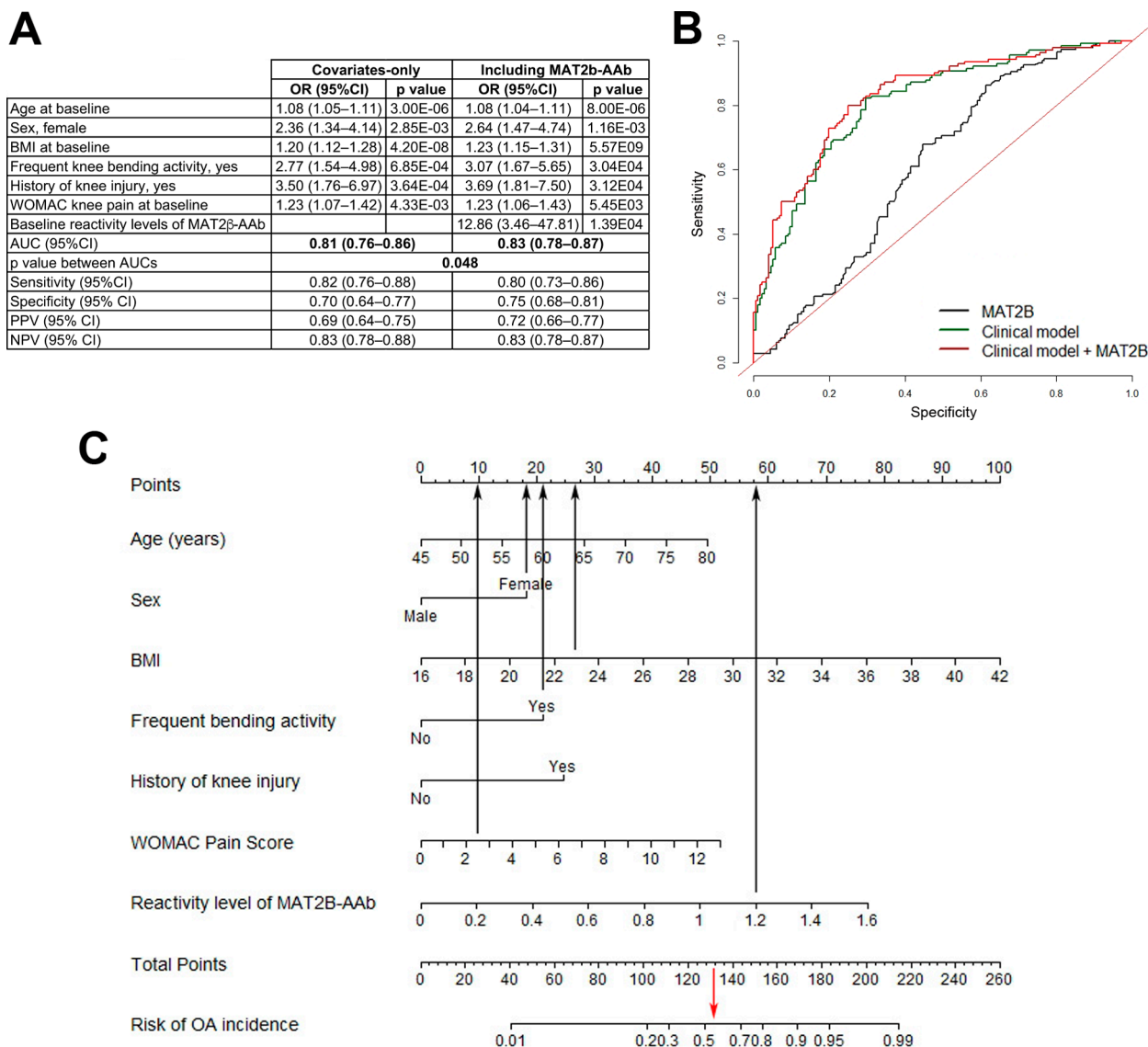
DISCUSSION

One of the features of a prognostic marker is the ability to predict the future occurrence of a certain disease among people who do not have it.[15] The production of antibodies against self-proteins is a characteristic feature of many diseases.[16] Considering the fact that AAbs can often be detected at asymptomatic stages,[4] they might have the potential to identify susceptible individuals or populations and facilitate prognosis. The idea that AAbs can be used to predict a disease state has been extensively studied in different disorders, such as cancer[17–19] or type 1 diabetes.[20 21] In the field of rheumatic diseases, AAbs have a fundamental value in the diagnosis of those with an autoimmune pathogenesis, such as systemic lupus erythematosus[22] and rheumatoid arthritis.[23]

Although OA is not considered an autoimmune disorder, the immune system is highly related with early disease.[24] However, existing literature related to the presence of AAbs in patients with OA is limited.[8 25–27] Indeed, this is the first study that evaluates the usefulness of AAbs to stratify patients with asymptomatic OA. The use of a large-scale approach (NAPPA) enabled the search of a massive number of putative AAbs, which is the largest screening performed to date in the OA field. With this approach, we have identified significant levels of reactivity from AAbs against six different proteins that are associated with the future incidence of the disease. At this point, a characteristic of this approach should be taken into consideration when interpreting the findings presented herein: The low sera dilutions employed in this work lead to the primary detection of class M immunoglobulins (IgM), which, in contrast to IgGs, have no immune memory. However, IgMs are not subjected to immunoregulation[28] and are formed early in the immune response. Therefore, specific antibodies of the IgM class might be important in the diagnosis of chronic diseases.[29]

Table 2 Characteristics at baseline of the study participants in the verification and replication phases

Covariates	Verification phase		Replication phase	
	Incident OA (n=146)	Non-incident OA (n=181)	Incident OA (n=65)	Non-incident OA (n=43)
Age, mean years (SD)	60.65 (8.51)	56.61 (8.57)	58.26 (9.40)	59.86 (8.50)
Sex, n (%) female	98 (67.1)	102 (56.4)	26 (60.5)	25 (38.5)
BMI, mean kg/m² (SD)	28.93 (4.59)	25.88 (4.14)	29.75 (4.94)	28.29 (3.69)
Frequent knee bending activity, n (%) yes	110 (75.3)	106 (58.6)	30 (71.4)	27 (41.5)
History of knee injury, n (%) yes	39 (26.7)	23 (12.7)	9 (21.4)	7 (10.9)
WOMAC pain score	1.63 (2.36)	0.61 (1.63)	3.21 (3.58)	1.68 (2.43)

BMI, body mass index; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

A

	Covariates-only		Including MAT2b-AAb	
	OR (95%CI)	p value	OR (95%CI)	p value
Age at baseline	1.08 (1.05–1.11)	3.00E-06	1.08 (1.04–1.11)	8.00E-06
Sex, female	2.36 (1.34–4.14)	2.85E-03	2.64 (1.47–4.74)	1.16E-03
BMI at baseline	1.20 (1.12–1.28)	4.20E-08	1.23 (1.15–1.31)	5.57E09
Frequent knee bending activity, yes	2.77 (1.54–4.98)	6.85E-04	3.07 (1.67–5.65)	3.04E04
History of knee injury, yes	3.50 (1.76–6.97)	3.64E-04	3.69 (1.81–7.50)	3.12E04
WOMAC knee pain at baseline	1.23 (1.07–1.42)	4.33E-03	1.23 (1.06–1.43)	5.45E03
Baseline reactivity levels of MAT2β-AAb			12.86 (3.46–47.81)	1.39E04
AUC (95%CI)	**0.81 (0.76–0.86)**		**0.83 (0.78–0.87)**	
p value between AUCs	**0.048**			
Sensitivity (95%CI)	0.82 (0.76–0.88)		0.80 (0.73–0.86)	
Specificity (95% CI)	0.70 (0.64–0.77)		0.75 (0.68–0.81)	
PPV (95% CI)	0.69 (0.64–0.75)		0.72 (0.66–0.77)	
NPV (95% CI)	0.83 (0.78–0.88)		0.83 (0.78–0.87)	

B

C

Figure 3 Prognostic model for incident radiographic KOA. (A) Metrics comparing the covariates-only model with the biomarker plus covariates model. (B) ROC curve for the models. (C) Nomogram of the biomarker plus covariates prognostic model. To use the nomogram, a straight edge on the top of the figure identifies the value on the points scale that corresponds to the score for each predictor (black arrows pointing up). In addition, the straight edge is aligned with the total points to determine probability at the bottom of the nomogram, once all the points for each predictor are summed. For example, a woman (18 points) of 45-year old (0 points), with a BMI of 23 kg/m^2 (27 points), who is involved in an activity with frequent knee bending (21 points), with no history of injury (0 points), a WOMAC pain score of 2.5 (10 points), and whose reactivity levels of MAT2β-AAb in serum were 1.2 a.u. (57.5 points) renders a total of 133.5 points. This value gives her a probability of 55.5% to develop radiographic KOA within a period of 96 months (red arrow pointing down). a.u., arbitrary units; AUC, area under the curve; BMI, body mass index; KOA, knee osteoarthritis; MAT2β-AAb, methionine adenosyltransferase two beta-autontibody; NPV, negative predictive value; OA, osteoarthritis; PPV, positive predictive value; ROC, receiver operating characteristic; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Table 3 Predictive capacity of the MAT2β-AAb plus covariates model in the verification and replication phases

	Verification phase	Replication phase	P value between AUCs
AUC (95% CI)	**0.83 (0.78 to 0.87)**	**0.76 (0.66 to 0.86)**	**0.218**
Sensitivity % (95% CI)	80 (73 to 86)	71 (56 to 83)	
Specificity % (95% CI)	75 (68 to 81)	81 (70 to 91)	
PPV % (95% CI)	72 (66 to 77)	71 (60 to 83)	
NPV % (95% CI)	83 (77 to 87)	81 (74 to 89)	

AUC, area under the curve; MAT2β-AAb, methionine adenosyltransferase two beta-autontibody; NPV, negative predictive value; PPV, positive predictive value.

The results for MAT2β-AAb have been verified on 327 individual samples at baseline from the OAI cohort, which provides a robust evaluation of its ability to classify patients at baseline as incident or non-incident during a 96-month period. Moreover, KM curves showed a significant association of the baseline reactivity levels of MAT2β-AAb with the time of KOA appearance. This statistical approach has been widely used in cancer biomarkers[30–32] and it has been recently introduced in the rheumatology field.[33 34] Interestingly, our results showed that higher baseline reactivity levels of this AAb result in a sooner development of radiographic KOA.

MAT2β is the regulatory subunit responsible of enhancing or inhibiting the synthesis of SAMe. This latter compound plays

a vital role in methylation, transsulfuration and aminopropylation pathways,[35] and it has been employed as dietary supplement for OA management.[35–38] Although there is no evidence of the direct involvement of MAT2β in OA, its fundamental role in key biological processes for the pathogenesis of this disease (online supplementary figure S2) turns it into a potential marker of interest. Curiously, this protein was not included in either the planar arrays or the NAPPA developed previously by our group for the screening of OA-associated AAb.[8]

The MAT2β-AAb-only model showed a modest ability to predict radiographic KOA development, yielding an AUC of 0.62 with 39% specificity and 86% sensitivity. In the OA field, this modest predictive capacity is in agreement with that obtained for different biomarkers that have been evaluated in the last years to predict relevant OA progression. For example, Eckstein and collaborators examined the relationship of 15 molecular markers with structural progression based on femorotibial cartilage loss.[39] The strongest predictors of longitudinal thinning were serum C-terminal telopeptide of collagen type I (CTX-I) and plasma N-terminal propeptide of type II procollagen, yielding AUCs of 0.65 and 0.64, respectively, but all the remaining biomarkers showed AUCs<0.60. Furthermore, in the meta-analysis published by Valdes and collaborators,[40] urine CTX-II also showed a limited predictive capacity (AUC ≤0.63). Recently, Kraus and collaborators have investigated a target set of 18 biochemical markers (baseline and time-integrated concentrations (TICs) over 12 and 24 months) as predictors of symptomatic and radiographic KOA progression.[41] Among all of them, the best single biomarker was the 24 M TIC CTX-II measured in urine, yielding an AUC=0.58.

With the aim of defining a useful non-radiographic prognostic model focused on KOA, different non-radiographic clinical factors related with risk of incident KOA in the literature[42–44] have been analysed in this study by univariable logistic regression analysis to look for significant predictors. It is important to specify that this study is based on a Caucasian US population, which may not comprise all the factors that enhance predisposition to OA. Among the clinical variables finally included in the model, the history of knee injury showed the highest OR, which was markedly lower than the reactivity levels of MAT2β–AAb (OR 2.57 vs 5.99) for being incident. As shown herein, the use of stepwise multivariable regression analysis resulted in one prognostic model of KOA incidence with an AUC=0.81. Using data from individuals in the Rotterdam study, a prediction model using clinical factors that yields an AUC of 0.66 was defined.[45] In another study, Zhang and collaborators defined a model of incidence of radiographic KOA with data from the Nottingham cohort, the OAI cohort and the Genetics of Osteoarthritis and Lifestyle (GOAL) study.[46] This model, including variables such as age, gender, BMI, occupational risk, family history and knee injury yielded the greatest AUC (0.74) in the GOAL population, compared with the OAI (AUC=0.60) and the Nottingham (AUC=0.69). In our case, the remarkably high ability to predict the appearance of radiographic KOA using this covariates-only model could be due to the high prevalence of the disease in our verification cohort (45% of incident participants). Indeed, the application of this model into the whole OAI database yielded a lower AUC (AUC <0.70 (data not shown)).

Despite the rather low AUC of MAT2β-AAb, the addition of this potential biomarker to the prognostic covariates-only model led to an increase in its discriminative ability (AUC=0.83), being this increase statistically significant (p=0.048). A similar improvement, but in this case not significant, was previously reported by including uCTX-II levels in a clinical prediction model for KOA.[45] At this point, it is important to take into account that among all the patients involved in this study, 42.5% of them had already developed radiographic OA in the off-target knee (KL ≥2). Nevertheless, we observed that the prognostic ability of MAT2β-AAb levels is maintained after removing subjects with contralateral OA at baseline (AUC=0.61 (0.54–0.68)). In addition, the inclusion of the presence of contralateral OA to the proposed MAT2β-AAb plus covariates model did not improve its predictive capacity (p=0.093). This strengthens the utility of this biomarker to predict incidence of KOA without the need of any radiographical information from the patients, avoiding their exposure to harmful radiation. Finally, the prognostic model combining baseline levels of MAT2β-AAb with clinical variables was replicated in an independent population, which confirms its putative utility to predict the appearance of radiographic KOA and strongly suggests it can be generalisable to the wider population.

In summary, the present study shows that a high antibody reactivity against MAT2β protein in serum is associated at baseline with individuals who will develop incident radiographic KOA, and also with an earlier appearance of the disease. Our results suggest that the inclusion of MAT2β-AAb in a clinical prognostic model for radiographic KOA could improve the identification of individuals who will develop the disease before 96 months.

Author affiliations
[1]Grupo de Investigación de Reumatología, Unidad de Proteomica, INIBIC-Complejo Hospitalario Universitario A Coruña, SERGAS, Universidad de A Coruña, A Coruña, Spain
[2]Grupo de Epidemiología Clínica y Bioestadística, INIBIC-Complejo Hospitalario Universitario A Coruña, SERGAS, Universidad de A Coruña, A Coruña, Spain
[3]Grupo de Investigacion de Reumatologia, Unidad de Genomica, INIBIC-Complexo Hospitalario Universitario de A Coruña, SERGAS, Universidad de A Coruña, A Coruña, Spain
[4]Virginia G. Piper Center for Personalized Diagnostics, Biodesign Institute-Arizona State University, Tempe, Arizona, USA
[5]Department of Medicine and General Cytometry Service-Nucleus. Proteomics Unit. CIBER-ONC, Cancer Research Center (IBMCC/CSIC/USAL/IBSAL), Salamanca, Spain
[6]Grupo de Investigacion Reumatologia, Unidad de Investigacion Clinica, INIBIC-Complejo Hospitalario Universitario A Coruña, SERGAS, Universidad de A Coruña, A Coruña, Spain
[7]Grupo de Investigacion de Reumatologia, INIBIC-Complejo Hospitalario Universitario A Coruña, SERGAS, Departamento de Medicina, Universidad de A Coruña, A Coruña, Spain

Osteoarthritis

ORCID iDs
Cristina Ruiz-Romero http://orcid.org/0000-0001-7649-9803
Francisco J Blanco http://orcid.org/0000-0001-9821-7635

REFERENCES

1 Sen R, Hurley JA. Osteoarthritis. *StatPearls* 2018.
2 Kraus VB, Blanco FJ, Englund M, *et al*. Call for standardized definitions of osteoarthritis and risk stratification for clinical trials and clinical use. *Osteoarthritis Cartilage* 2015;23:1233–41.
3 Geurts J, Jurić D, Müller M, *et al*. Novel ex vivo human osteochondral explant model of knee and spine osteoarthritis enables assessment of inflammatory and drug treatment responses. *Int J Mol Sci* 2018;19:1314.
4 Leslie D, Lipsky P, Notkins AL. Autoantibodies as predictors of disease. *J Clin Invest* 2001;108:1417–22.
5 Macdonald IK, Parsy-Kowalska CB, Chapman CJ. Autoantibodies: opportunities for early cancer detection. *Trends Cancer* 2017;3:198–213.
6 Katchman BA, Chowell D, Wallstrom G, *et al*. Autoantibody biomarkers for the detection of serous ovarian cancer. *Gynecol Oncol* 2017;146:129–36.
7 Wang H, Demirkan G, Bian X, *et al*. Identification of Antibody Against SNRPB, Small Nuclear Ribonucleoprotein-Associated Proteins B and B', as an Autoantibody Marker in Crohn's Disease using an Immunoproteomics Approach. *J Crohns Colitis* 2017;11:848–56.
8 Henjes F, Lourido L, Ruiz-Romero C, *et al*. Analysis of autoantibody profiles in osteoarthritis using comprehensive protein array concepts. *J Proteome Res* 2014;13:5218–29.
9 Anderson KS, Sibani S, Wallstrom G, *et al*. Protein microarray signature of autoantibody biomarkers for the early detection of breast cancer. *J Proteome Res* 2011;10:85–96.
10 Ramachandran N, Raphael JV, Hainsworth E, *et al*. Next-Generation high-density self-assembling functional protein arrays. *Nat Methods* 2008;5:535–8.
11 Rego-Pérez I, Blanco FJ, Roemer FW, *et al*. Mitochondrial DNA haplogroups associated with MRI-detected structural damage in early knee osteoarthritis. *Osteoarthritis Cartilage* 2018;26:1562–9.
12 Halilaj E, Le Y, Hicks JL, *et al*. Modeling and predicting osteoarthritis progression: data from the osteoarthritis initiative. *Osteoarthritis Cartilage* 2018;26:1643–50.
13 Bian X, Wallstrom G, Davis A, *et al*. Immunoproteomic profiling of antiviral antibodies in new-onset type 1 diabetes using protein arrays. *Diabetes* 2016;65:285–96.
14 Wang J, Barker K, Steel J, *et al*. A versatile protein microarray platform enabling antibody profiling against denatured proteins. *Proteomics Clin Appl* 2013;7:378–83.
15 Matson E, Baliog C, Reginato AM. Papel de Los biomarcadores en La artrosis. in: Artrosis Fisiopatología, diagnóstico Y tratamiento. *Editorial Médica Panamericana; Madrid* 2010;21:327–53.
16 Gibson DS, Qiu J, Mendoza EA, *et al*. Circulating and synovial antibody profiling of juvenile arthritis patients by nucleic acid programmable protein arrays. *Arthritis Res Ther* 2012;14.
17 Qi S, Huang M, Teng H, *et al*. Autoantibodies to chromogranin A are potential diagnostic biomarkers for non-small cell lung cancer. *Tumor Biol* 2015;36:9979–85.
18 Chen H, Werner S, Tao S, *et al*. Blood autoantibodies against tumor-associated antigens as biomarkers in early detection of colorectal cancer. *Cancer Lett* 2014;346:178–87.
19 Kunizaki M, Hamasaki K, Wakata K, *et al*. Clinical value of serum p53 antibody in the diagnosis and prognosis of esophageal squamous cell carcinoma. *Anticancer Res* 2018;38:1807–13.
20 Bonifacio E, Genovese S, Braghi S, *et al*. Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity. *Diabetologia* 1995;38:816–22.
21 Fabris M, Zago S, Liguori M, *et al*. Anti-zinc transporter protein 8 autoantibodies significantly improve the diagnostic approach to type 1 diabetes: an Italian multicentre study on paediatric patients. *Autoimmun Highlights* 2015;6:17–22.
22 Putterman C, Wu A, Reiner-Benaim A, *et al*. SLE-key® rule-out serologic test for excluding the diagnosis of systemic lupus erythematosus: developing the ImmunArray iCHIP®. *J Immunol Methods* 2016;429:1–6.
23 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569–81.
24 Ene R, Sinescu RD, Ene P, *et al*. Synovial inflammation in patients with different stages of knee osteoarthritis. *Rom J Morphol Embryol* 2015;56:169–73.
25 Jasin HE. Autoantibody specificities of immune complexes sequestered in articular cartilage of patients with rheumatoid arthritis and osteoarthritis. *Arthritis Rheum* 1985;28:241–8.
26 Du H, Masuko-Hongo K, Nakamura H, *et al*. The prevalence of autoantibodies against cartilage intermediate layer protein, YKL-39, osteopontin, and cyclic citrullinated peptide in patients with early-stage knee osteoarthritis: evidence of a variety of autoimmune processes. *Rheumatol Int* 2005;26:35–41.
27 Ruthard J, Hermes G, Hartmann U, *et al*. Identification of antibodies against extracellular matrix proteins in human osteoarthritis. *Biochem Biophys Res Commun* 2018;503:1273–7.
28 Diaz-Zaragoza M, Hernandez-Avila R, Viedma-Rodriguez R, *et al*. Natural and adaptive IgM antibodies in the recognition of tumor-associated antigens of breast cancer (review). *Oncol Rep* 2015;34:1106–14.
29 Burrell CJ, Howard CR, Murphy FA. Adaptive Immune Responses to Infection. In: *Fenner and White's Medical Virology*. 5th edn. Academic Press (ELSERVIER), 2017: 65–76.
30 Albertus DL, Seder CW, Chen G, *et al*. AZGP1 autoantibody predicts survival and histone deacetylase inhibitors increase expression in lung adenocarcinoma. *J Thorac Oncol* 2008;3:1236–44.
31 Aguirre-Gamboa R, Gomez-Rueda H, Martínez-Ledesma E, *et al*. SurvExpress: an online biomarker validation tool and database for cancer gene expression data using survival analysis. *PLoS One* 2013;8:e74250.
32 Rinaldetti S, Wirtz R, Worst TS, *et al*. Foxm1 predicts disease progression in non-muscle invasive bladder cancer. *J Cancer Res Clin Oncol* 2018;144:1701–9.
33 Lezcano-Valverde JM, Salazar F, León L, *et al*. Development and validation of a multivariate predictive model for rheumatoid arthritis mortality using a machine learning approach. *Sci Rep* 2017;7.
34 van Wulfften Palthe AFY, Clement ND, Temmerman OPP, *et al*. Survival and functional outcome of high tibial osteotomy for medial knee osteoarthritis: a 10–20-year cohort study. *Eur J Orthop Surg Traumatol* 2018;28:1381–9.
35 Hosea Blewett HJ. Exploring the mechanisms behind S-adenosylmethionine (same) in the treatment of osteoarthritis. *Crit Rev Food Sci Nutr* 2008;48:458–63.
36 Soeken KL, Lee WL, Bausell RB, *et al*. Safety and efficacy of S-adenosylmethionine (same) for osteoarthritis. *J Fam Pr* 2002;51:425–30.
37 Najm WI, Reinsch S, Hoehler F, *et al*. S-adenosyl methionine (SAMe) versus celecoxib for the treatment of osteoarthritis symptoms: a double-blind cross-over trial. [ISRCTN36233495]. *BMC Musculoskelet Disord* 2004;5:6.
38 Kim J, Lee EY, Koh E-M, *et al*. Comparative clinical trial of S-adenosylmethionine versus nabumetone for the treatment of knee osteoarthritis: an 8-week, multicenter, randomized, double-blind, double-dummy, phase IV study in Korean patients. *Clin Ther* 2009;31:2860–72.
39 Eckstein F, Le Graverand MPH, Charles HC, *et al*. Clinical, radiographic, molecular and MRI-based predictors of cartilage loss in knee osteoarthritis. *Ann Rheum Dis* 2011;70:1223–30.
40 Valdes AM, Meulenbelt I, Chassaing E, *et al*. Large scale meta-analysis of urinary C-terminal telopeptide, serum cartilage oligomeric protein and matrix metalloprotease degraded type II collagen and their role in prevalence, incidence and progression of osteoarthritis. *Osteoarthritis and Cartilage* 2014;22:683–9.
41 Kraus VB, Collins JE, Hargrove D, *et al*. Predictive validity of biochemical biomarkers in knee osteoarthritis: data from the FNIH oa biomarkers Consortium. *Ann Rheum Dis* 2017;76:186–95.
42 Nevitt M, Felson DT, Lester G. The osteoarthritis initiative. protocol for the cohort study. Available: https://oai.epi-ucsf.org/datarelease/docs/studydesignprotocol.pdf
43 Johnson VL, Hunter DJ. The epidemiology of osteoarthritis. *Best Pract Res Clin Rheumatol* 2014;28:5–15.
44 Sharma L, Hochberg M, Nevitt M, *et al*. Knee tissue lesions and prediction of incident knee osteoarthritis over 7 years in a cohort of persons at higher risk. *Osteoarthritis Cartilage* 2017;25:1068–75.
45 Kerkhof HJM, Bierma-Zeinstra SMA, Arden NK, *et al*. Prediction model for knee osteoarthritis incidence, including clinical, genetic and biochemical risk factors. *Ann Rheum Dis* 2014;73:2116–21.
46 Zhang W, McWilliams DF, Ingham SL, *et al*. Nottingham knee osteoarthritis risk prediction models. *Ann Rheum Dis* 2011;70:1599–604.