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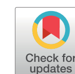
Utility of circulating serum miRNA profiles to evaluate the potential risk and severity of immune-mediated inflammatory disorders



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ABSTRACT

Immune-mediated inflammatory disorders (IMID) are a group of diseases that present inflammation as a major pathogenic mechanism. They affect 15% of the population and pose a heavy socio-economic burden. Despite the growing knowledge on the etiopathogenesis of these diseases and the marked improvement in their management, there is a lack of predictive markers of IMID development or severity suitable for early diagnosis and adjustment of treatment intensity. The possibility that certain circulating miRNA profiles could be used as biomarkers of risk of development and/or severity of several autoimmune diseases has fuelled the interest in using them to improve the selection of successful treatments. The multi-pronged approach proposed here sought to reveal circulating miRNAs and miRNA signatures that could act as new predictive biomarkers of IMID development and severity. Our results showed that the circulating levels of miR-19b and miR-26b were significantly decreased ($p < 0.001$) in IMID patients compared to controls. Furthermore, receiver operating characteristic (ROC) curve analysis showed that these miRNAs were suitable discriminators capable to identify an IMID, with areas under the curve (AUC) of 0.85 and 0.83, respectively. In addition, we established that miR-19a and miR-143 were significantly increased in IMID patients with severe disease ($p < 0.05$). In summary, our findings identify two different miRNA signatures. One of them is associated with the presence of IMIDs and could lead to the development of tools for their early detection. The second signature is able to discriminate between mild and severe forms of these disorders and could be a putative tool to select patient candidates for a more intense treatment.

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1. Introduction

One of the most important characteristics of the immune system is tolerance, which is the ability to recognise self-antigens and avoid immune reaction against them. Immune tolerance is maintained by the recognition of exposed self-antigens and by the inactivation or deletion of autoreactive T and B lymphocytes. Autoimmune diseases result from a breakdown of these central tolerance mechanisms, leading to T and B cell-mediated tissue damage [1].

Immune-mediated inflammatory disorders (IMIDs) are a group of chronic diseases that share an immune component characterised by undesired activation of the immune system, and present inflammation as a major pathogenic mechanism. Examples of IMIDs include psoriasis (Ps), rheumatoid arthritis (RA), Graves' disease (GD), and Graves' ophthalmopathy (GO), among others [2–6]. Collectively, their incidence and prevalence has markedly increased over the last decades affecting nearly 15% of the general population (Ps, 1–2%; RA, 0.5%–1%; GD, 1–2%). These diseases produce an enormous socio-economic burden due not only to medical costs, but also to progressive severe disability, influencing productivity and dependence of affected subjects [7–9]. The total costs of these disorders have been estimated to range from 1000 to 12,000€ per patient/year depending on the disease. In addition, there is an increased risk of early death due to cardiovascular complications and other co-morbidities [2,7,10,11]. The pathogenesis of IMIDs is complex and they are considered as multifactorial diseases. It is widely accepted that interactions between genetic background, immunity and the environment contribute to the breakdown of tolerance and the development of autoimmunity characteristic of IMIDs. Lately, a common goal in the management of IMIDs is to develop early diagnosis methods and tailored treatments for individual patients according to the severity of their disease. The identification of novel biomarkers that could be used as potential therapeutic targets for autoimmune diseases entails searching for new tools to reverse the tolerance break and adjust treatment intensity.

MicroRNAs (miRNAs) are a class of non-coding RNA molecules (19–25 nucleotides) that regulate gene expression at the post-transcriptional level inhibiting mRNA translation or promoting its degradation [12,13]. By targeting a plethora of mRNAs, miRNAs can simultaneously regulate multiple pathways and biological processes in which one miRNA may regulate different genes while a certain gene can be regulated by different miRNAs. Hence, it is difficult to discern the specific function/effect of a specific miRNA. Studies using conditional transgenic mouse models for genes involved in the miRNA biogenesis pathway have provided data about the central role of miRNAs in the regulation of the immune response [14–16]. MiRNAs fine-tune the expression of genes involved in the immune response, and dysregulation of this process can lead to aberrant expression of different cytokines, and therefore to inflammation and loss of immune tolerance to self-antigens, which are a hallmark of common chronic inflammatory diseases [17–19]. Since miRNAs can be detectable in body fluids, such as serum or plasma, they have emerged as potentially useful biomarkers for risk assessment, diagnosis and prognosis [20]. Although the way in which an extracellular miRNA profile affects cell responses is hard to establish, data suggest that circulating miRNAs could be very useful as prognostic or diagnostic biomarkers for autoimmune diseases and of great importance in the design of future biological therapies [19,21].

Specific profiles of circulating miRNAs have been associated with different systemic autoimmune diseases including systemic lupus erythematosus, systemic sclerosis, Ps, RA and autoimmune thyroid disease (AITD) [19,22–24]. However, in order to unravel miRNAs most consistently associated with dysregulation of the immune system leading to autoimmune disorders, we have studied the expression of immunologically relevant miRNAs in serum samples from patients with different IMIDs, as well as its possible correlation with disease severity. From these data we have developed a signature model which can contribute to an improvement in the detection of severe IMID forms and/or to the identification of miRNAs as possible targets for drugs used to treat IMIDs. A major strength of this research is that we have searched for markers that may not only be specific of each IMID, but that also reveal common events present in several IMIDs, thereby studying possible links between the different types of diseases.

2. Material and methods

2.1. Patients and sample collection

The BIOIMID project was approved by the Clinical Research Ethics Committee of Hospital de la Princesa (CEIm Hospital Universitario La Princesa, PI-734) and written informed consent was obtained from all patients prior to inclusion, in accordance with the Declaration of Helsinki.

A total of 78 subjects were studied: 20 healthy subjects, 19 patients (18 females) diagnosed of GD with or without GO defined by the EUGOGO (European Group on Graves Ophtalmopathy) criteria [25], 19 patients (14 female) classified as RA by the 2010 EULAR/ACR (European League Against Rheumatism/American College of Rheumatology) criteria [26] and 20 (9 female) with Ps. The main clinical features of patients and controls are shown in Tables 1 and 2.

Patients were classified as having mild or severe disease according the following parameters for each group:

A) In the GO group, a cut-off value of 3 in the 7 point scale of the clinical activity score (CAS) was used to diagnose active GO [25]. A total of 9 patients had mild disease, and 10 patients had severe disease with an active GO.

B) In the RA group, the 28-joint disease activity score (DAS28) was used to asses severity; 9 patients were classified as having mild disease (DAS28 < 2.6) and 10 as having severe disease (DAS28 > 5.1) [27].

C) In the Ps group severity was assessed using Psoriasis Area Severity Index (PASI); 9 patients with PASI < 10 were classified as having mild disease and 11 patients with PASI ≥ 10 as having severe disease.

Blood samples were collected before treatment prescription. Serum was obtained by 10 min centrifugation ($1500 \times g$) of 10 ml of total blood and stored at -80 °C until use at the Biobank of Instituto de Investigación Sanitaria Princesa.

2.2. RNA extraction and RT-PCR

Serum miRNA isolation was performed as previously described [22]. To test haemolysis in serum samples, the absorbance of free haemoglobin at 414 nm was measured using a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific), samples with a peak > 0.2 were discarded. RNA was purified from 200 µl serum samples using miRCURY RNA Isolation Kit Biofluids (Exiqon), according to manufacturer's instruction. Briefly, lysis solution containing 1.25 µg/ml of MS2 bacteriophage RNA (Roche Diagnostics) and the RNA spike-in

Table 1
Clinical features of patients with IMID classified according to disease severity.

	Mild	Severe	Controls	p-value
n	27	31	18	–
Gender (F/M)	20/7	21/10	9/9	0.105
Age (yr)	51.9 (34.2–64.3)	49.66 (35–61.3)	44.5 (27.7–56.7)	0.4681
Disease duration (m)	2.4 (0.4–97.2)	72 (2.6–174)	NA	0.0698
Smokers (YES/NO/EX)	8/13/6	8/19/4	5/12/1	0.953
BMI	26.1 (23.92–28.4)	25.56 (21.72–31.05)	27.2 (22.2–29.7)	0.9481

Values show number for categorical values and median (interquartile intervals) for continuous variables. F/M = female/male. BMI: Body Mass Index.

Table 2
Clinical features of patients with Graves' disease, Rheumatoid Arthritis and Psoriasis classified according to disease severity.

	GD		RA		Psoriasis	
	Mild	Severe	Mild	Severe	Mild	Severe
Gender (F/M)	9	9/1	5/4	9/1	6/3	3/8
Age (yr)	51 (40–73)	50 (33–58)	52.8 (34.3–54.1)	49.9 (34–62.1)	53 (42–68.5)	40 (32–45.5)
Disease Duration (months)	1.57 (0.86–4.1)	2.6 (0.3–5)	8 (3.9–16.9)	4.2 (3.1–6.3)	84 (0–156)	132 (96–198)
Smokers (YES/NO/EX)	2/6/1	4/5/1	4/1/4	0/7/3	2/6/1	5/3/3
BMI	24.5 (21.4–25.9)	21.9 (20.2–27.6)	26.1 (24.3–28.1)	29.4 (25.6–32.8)	27.5 (22.5–31.7)	26.5 (22.2–30.4)
Ophthalmopathy	0	10	–	–	–	–
TSH mU/mL	0 (0–9.2)	0 (0–7.2)	–	–	–	–
T4 ng/dL	2.03 (0.47–4.8)	1.54 (0.56–4.7)	–	–	–	–
Tg-Ab UI/mL	20 (20–1076)	133 (20–1292)	–	–	–	–
TPO-Ab UI/mL	20 (20–565.5)	421 (20–2409)	–	–	–	–
TSHR-Ab U/L	1.81 (1.38–3.24)	3.9 (2.94–6.12)	–	–	–	–
DAS28	–	–	2.1 (1.7–2.4)	6.2 (5.5–6.9)	–	–
HAQ	–	–	0.4 (0.1–0.5)	1.6 (1.1–1.9)	–	–
FR IU/ml	–	–	102 (25–188)	34 (27–101)	–	–
CCP 50 IU/ml	–	–	1472 (406–2070)	390.5 (327–498)	–	–
PASI					8.4 (6.2–9)	22.5 (16.45–35)

Values show number for categorical values and median (25–75 interquartile intervals) for continuous variables. GD (Graves' disease); RA (rheumatoid arthritis); F/M = female/male; TSH, thyrotropin (Normal range = 0.27–4.20); T4, thyroxine (Normal range = 0.93–1.7); Tg-Ab, anti-thyroglobulin antibody (Negative < 344); TPO-Ab, anti-thyroid peroxidase antibody (Negative < 100); TSHR-Ab, anti-thyrotropin receptor antibody (Negative < 0.7). DAS28: 28-joint disease activity score; HAQ: score from the Health Assessment Questionnaire (Spanish version) [64]; RF: Rheumatoid Factor (RF, assessed by nephelometry; positive > 20 IU/ml); CCP: *anti*-citrullinated peptide antibody (CCP, measured by enzyme immunoassay [EIA]: Immunoscan RA, Euro- Diagnostica; positive > 50 IU/ml); BMI: Body Mass Index. PASI: Psoriasis Area Severity Index.

templates UniSp2, UniSp4 and UniSp5 was added to 200 µl of serum. RNA was purified on microRNA mini spin columns and stored at −80 °C. The robustness of RNA isolation process was assessed using miRCURY microRNA QC Panels (Exiqon). First-strand cDNA was generated using cDNA synthesis kit (Exiqon) and subsequent quantitative Real Time PCR (q-RT-PCR) was performed in triplicates using microRNA LNA™ PCR primer sets (Exiqon). PCR was carried out in 384 well plates in a CFX384 Real-Time System (Roche). Samples from all groups within an experiment were processed at the same time.

2.3. RT-PCR analysis

Based on their role in the immune response or dysregulated expression in autoimmune diseases we selected a group of miRNAs with potential predictive value in the assessment of IMID severity including: hsa-miR-10a, hsa-mir-19a-3p, hsa-miR-19b-3p, hsa-mir-21-5p, miR-23a-3p, hsa-miR-26b-5p, hsa-miR-27a-3p, hsa-miR-29a-3p, hsa-miR-93-3p, hsa-miR-101-3p, hsa-mir-125b-5p, hsa-miR-126-3p, hsa-miR-127-3p, hsa-mir-142-3p, hsa-miR-143-3p, hsa-miR-146a-5p, hsa-miR-150-5p, hsa-miR-155-5p, hsa-mir-191-5p, hsa-miR-210-3p, hsa-mir-326, hsa-miR-451 (Supplementary Table 1). In addition, hsa-miR-103a-3p and hsa-miR-30c-5p were included as possible normalisers [28]. Relative Quantities (RQs) were determined using the log base 2 values of the Ct difference between miRNAs and UniSp2 spike-in. RQ values were then normalised using the value of mir-103a expression (NRQ). Identification of miR-103a as the more stable reference miRNA was carried out as previously described [29]. Briefly, stability score (SSS) was calculated for several miRNAs (Supplementary Table 2) by the combination of different algorithms, including geNorm [30], Normfinder [31] and Coefficient of Variation [29]. Regardless of groups, the expression of miR-10a, miR-127, miR-155 and miR-326 in serum was very low or even undetectable (Ct > 40, Supplementary Figure 1).

2.4. Statistical analysis

Descriptive results were expressed as mean ± standard deviation (SD), mean ± standard error of the mean (SEM), or median and 25–75 percentile, as appropriate. Pearson's bivariate correlations were performed for all quantitative variables. Differences between groups were compared using Chi-Square, Student T test or analysis of variance (ANOVA) as appropriate. A transformation of variable was applied to miRNA expression values to achieve a normal distribution in order to apply parametric tests.

A Pearson's correlation matrix analysis was performed between all miRNAs studied and the resulting pairwise correlation coefficients were depicted as a heatmap for visual evaluation. Correlation values were hierarchically clustered following Euclidean distances between objects and complete linkage using superheat package in R version 3.5.1 [32]. Differences for each normalised miRNA between controls and IMID groups were compared using a logistic regression model, adjusted by age and sex, as recommended in previous reports [33]. The optimal cut-off values of miRNA expression to determine low and high risk groups were assessed using the maxstat R package [34]. Finally, receiver operating characteristic (ROC) curve analyses were performed to assess the classification power of each adjusted logistic regression model for prediction of IMID or disease severity using pROC package in R version 3.5.1 [35]. The p-values were two sided, and statistical significance was considered when $p < 0.05$. Data are presented with the specific p-values: $p < 0.05$, $p < 0.01$ and $p < 0.001$. Box-plots were represented using GraphPad Prism 4 software.

3. Results

3.1. Clinical characteristics of IMID patients

To identify common or specific circulating miRNAs for different IMIDs, expression analysis was performed using RT-PCR assays in serum samples from patients with GD (n = 19), RA (n = 19) and psoriasis (n = 20), and from control subjects (n = 20). Clinical and demographic characteristics of patients are shown in Tables 1 and 2 A total of 27 patients had mild IMID whereas 31 had a severe disease. Regarding demographic differences, the number of females was higher in the IMID group because of the increased susceptibility of females for these diseases, whereas no differences were detected in age between patients with mild IMID (51.9 years, 34.2–64.3), severe IMID (49.66, 35–61.3) and controls (44.5, 27.7–56.7). Disease duration was slightly higher in the severe group, although the difference did not reach statistical significance. Smoking conditions and body mass index (BMI)

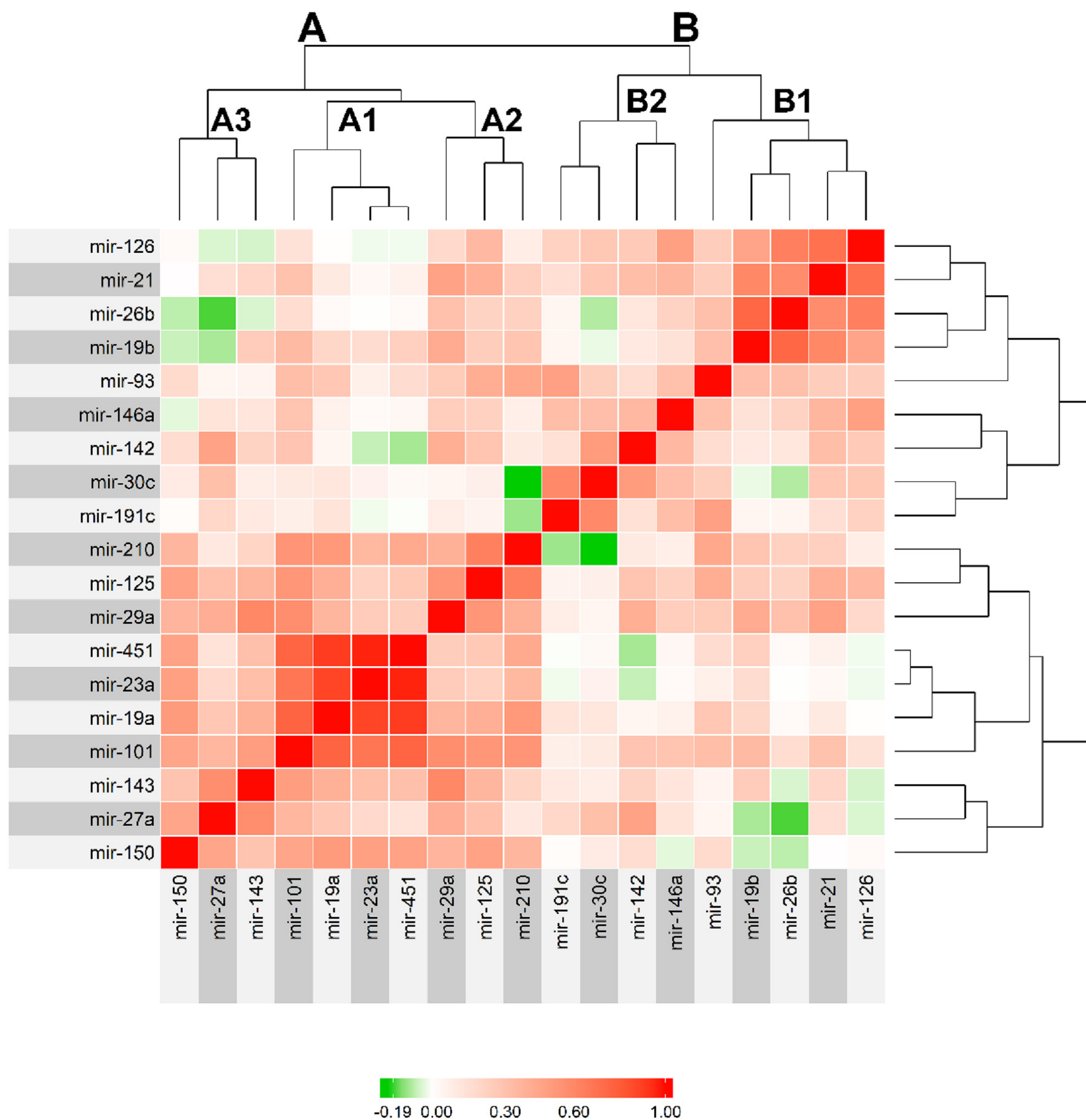


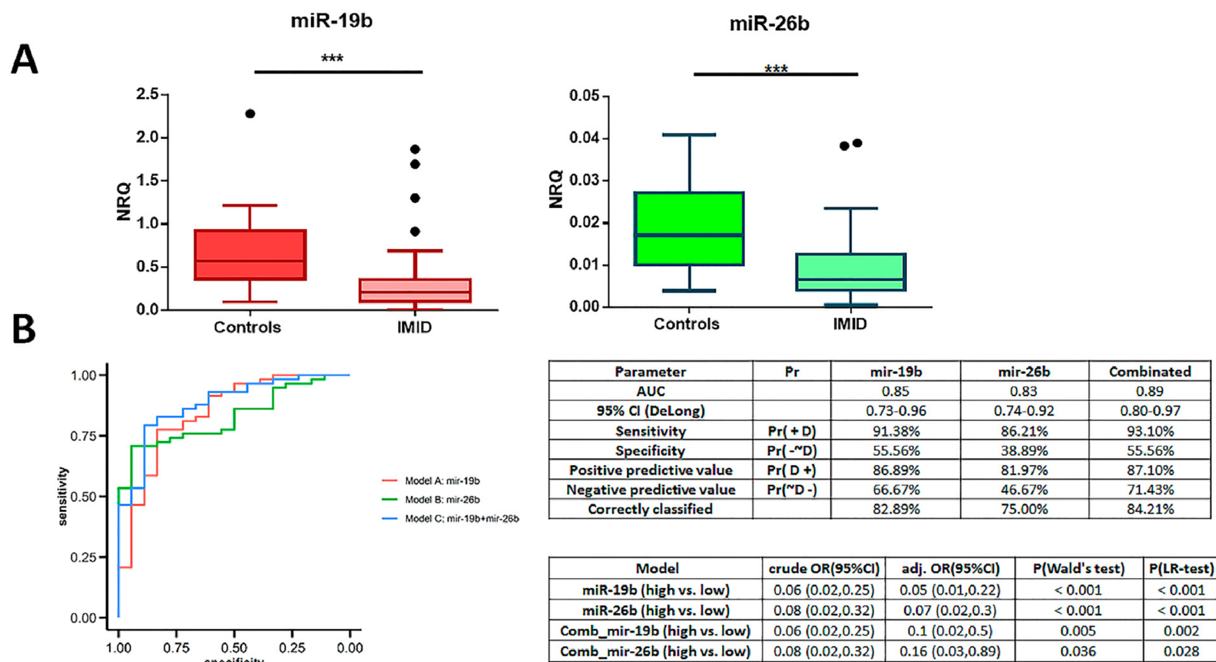
Fig. 1. Correlation map between miRNAs in IMIDs. Graphical display of Pearson's correlation matrix between normalised relative quantities of different miRNAs in serum samples from IMID patients. Correlation coefficient values (rho) arranged by hierarchical clustering are represented by a colour scale ranging from −0.19 (negative correlation, green) to 1 (positive correlation, red). The correlation matrix reveals two different clusters of miRNAs (A and B). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were also similar between groups. Then, the influence of clinical and demographic variables on miRNA expression levels was analysed in the control group, finding that 6 different miRNAs were significantly associated with age and sex, whereas there was no significant association between any of the miRNAs analysed and BMI (Supplementary Figure 2). Thus, subsequent analyses were adjusted by sex and age as previously reported [33].

3.2. Expression of miRNAs in IMID patients

To identify potential miRNA groups in serum samples based on their molecular expression profile, a Pearson's correlation matrix analysis was carried out between normalised relative quantities of miRNAs. Unsupervised hierarchical clustering showed two main clusters of miRNAs that behaved in a different manner according to the correlation of their expression profiles. Group A included miRNAs with homogeneous positive correlations, which could be divided in three subclusters: A1 (miR-101, miR-19a, miR-23a, and miR-451), A2 (miR-29a, miR-125 and miR-210) and A3 (miR-150, miR-27a and miR-143) (Fig. 1). Group B showed heterogeneous expression correlations and could be divided in 2 subclusters: B1 (miR-93, miR-19b, miR-26b, miR-21 and miR-126) and B2 (miR-191, miR-30c, miR-142, and miR-146a) (Fig. 1). These different expression profiles suggest that broad global commonalities may exist between miRNA groups related to their post-transcriptional regulation in IMID. Interestingly, group A tended to show none or negative expression correlations with miRNAs from group B.

In order to determine whether these different expression profiles had a functional relevance, we first analysed differential miRNA expression between IMID patients and healthy subjects. Our data showed that only the expression of the members of subcluster B1 miR-19b and miR-26b was significantly decreased ($p < 0.001$) in the IMID group compared with the control group (Fig. 2A and Supplementary Table 3). The best NRQs cut-off for IMID discrimination were 0.356 for miR-19b ($p = 0.0001$) and 0.0091 for miR-26b ($p = 0.0008$). ROC curve analyses for these cut-offs suggested that both miRNAs were moderate



Parameter	Pr	mir-19b	mir-26b	Combinated
AUC		0.85	0.83	0.89
95% CI (DeLong)		0.73-0.96	0.74-0.92	0.80-0.97
Sensitivity	Pr(+ D)	91.38%	86.21%	93.10%
Specificity	Pr(-~D)	55.56%	38.89%	55.56%
Positive predictive value	Pr(D +)	86.89%	81.97%	87.10%
Negative predictive value	Pr(~D -)	66.67%	46.67%	71.43%
Correctly classified		82.89%	75.00%	84.21%

Model	crude OR(95%CI)	adj. OR(95%CI)	P(Wald's test)	P(LR-test)
miR-19b (high vs. low)	0.06 (0.02,0.25)	0.05 (0.01,0.22)	< 0.001	< 0.001
miR-26b (high vs. low)	0.08 (0.02,0.32)	0.07 (0.02,0.3)	< 0.001	< 0.001
Comb_mir-19b (high vs. low)	0.06 (0.02,0.25)	0.1 (0.02,0.5)	0.005	0.002
Comb_mir-26b (high vs. low)	0.08 (0.02,0.32)	0.16 (0.03,0.89)	0.036	0.028

Fig. 2. Serum miRNAs associated with IMIDs. A) Expression (Normalised relative quantity, NRQ) of statistically significant miRNAs in serum samples from IMID patients compared to controls. Results were analysed by a logistic model adjusted by age and sex. Data are presented as box-plots *P = 0.05. **P = 0.01. ***P = 0.001. B) Receiver Operating Characteristic (ROC) curve analyses performed to assess the diagnostic value of circulating miR19b, miR26b and the combination of both to discriminate between controls and IMID patients. The table shows areas under the curve (AUC), percentages of correctly predicted cases, 95% confidence intervals (95% CIs), sensitivities, and specificities of the analyses. Results of both models are given as crude odds ratios (OR, 95% CI), and odds ratios adjusted by sex and age (adj. OR, 95% CI).

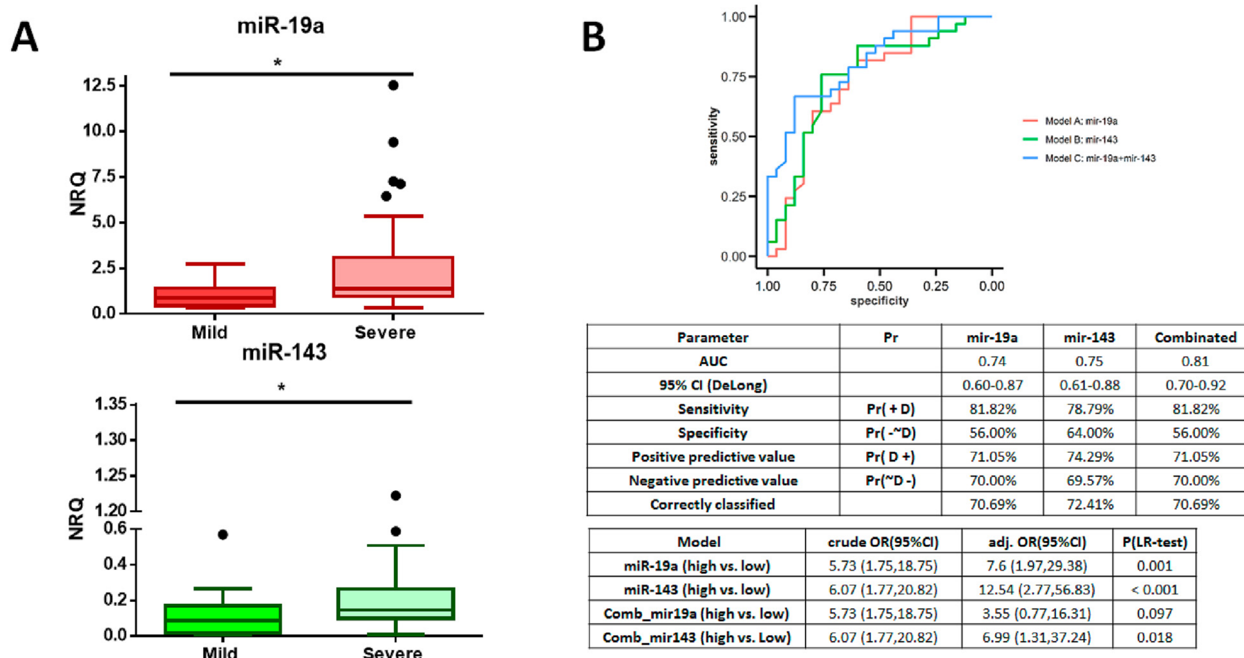
discriminators between patients with IMID and healthy donors, being the areas under the curve (AUC) 0.85 and 0.83 with high sensitivity 91.38% and 86.21% but low specificity 55.56% and 38.89, respectively (Fig. 2B). Subsequent analysis of both miRNAs showed that combining miR-19b and miR-26b in the regression model achieved the best predictive value for disease discrimination with an AUC of 0.89. Interestingly, increased levels of both biomarkers had a protective effect on the development of IMIDs [Odds Ratio (OR) = 0.05 and 95% confidence interval (95%CI):0.01–0.22 for miR-19b and OR = 0.07 (95%CI: 0.02–0.3) for miR-26b]. Logistic regression model parameters are summarised in Fig. 2B.

Finally, we analysed miRNA expression according to disease severity (mild and severe IMID). The multivariate logistic regression analysis revealed that expression of both the member of subcluster A1 miR-19a ($p < 0.05$) and the member of subcluster A3 miR-143 ($p < 0.05$) were significantly increased in patients with severe disease compared to patients with mild disease (Fig. 3A and Supplementary Table 4). AUCs for these miRNAs were 0.74 (95%CI: 0.6–0.87) and 0.75 (95%CI: 0.61–0.88), respectively (Fig. 3B). The best NRQs for discrimination of severity were 0.93 for miR-19a ($p = 0.05$) and 0.085 for miR-143 ($p = 0.04$). OR for these miRNAs were 7.6 and 12.54, respectively, thereby indicating that their increased expression represents a risk factor for developing a severe IMID. Interestingly, the composite of both parameters had a better predictive value with an AUC of 0.81 and a percentage of prediction of 70.69%. Logistic regression model parameters are summarised in Fig. 3B. Interestingly, miR-19a and miR-143 are co-clustered (converge) in a closely related group of miRNAs as shown in the global miRNA analysis (Fig. 1). These data suggest that the dysregulation of some miRNAs could be a common factor to develop a severe form of IMID.

4. Discussion

Accumulating evidence supports the use of miRNAs as prognostic biomarkers of disease and as a valuable diagnostic strategy [36]. MiRNAs are specific and stable, and they are present in serum, thus they could be useful as minimally invasive diagnostic tools for various diseases as well as for research on novel therapeutic targets [37]. Since miRNAs could play a role in maintaining immune tolerance and preventing autoimmunity, in the present study we have analysed immunologically relevant miRNAs in different IMIDs including GD, Ps and RA. A major strength of this research resides on the fact that we have searched for markers that may not only be specific of each disease, but that also reveal common events present in several IMIDs, thereby unveiling powerful, yet not obvious, links between the different types of IMIDs. This is a very innovative approach to diagnosis, as previous studies have usually focused on a specific IMID. In this sense, our results can have important implications in managing all these related disorders as a whole, since they would allow to evaluate the possibility of development of different IMIDs on a certain patient.

In this study we have unveiled a distinctive miRNA profile, characterised by significant downregulation of miR-19b and miR-26b, as a possible regulator of IMID development. MiR-19b is part of the miR-17-92 cluster, which regulates $CD4^+$ T-cell antigen responses and promotes Th17 responses [38–40]. Furthermore, miR-19b is downregulated in monocytes from patients with systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS), in which is important for the modulation of tissue factor expression [41]. MiR-26b has also been shown to play a role in inflammation and inflammatory cytokine secretion in RA and as an immune response modulator in osteoarthritis [42,43]. Furthermore, miR-26b targets the inflammatory factor prostaglandin-endoperoxide synthase 2 (PTGS2) which plays important roles in inflammatory diseases inducing the production of prostaglandin E2 (PGE2) [44,45]. Interestingly, these two miRNAs shared the same subcluster (B1) with miR-21 and miR-126, which suggests that their functions may be closely interrelated. Indeed, miR-21 has largely been associated with the development of autoimmune diseases, playing important roles in the immune system [46]. However,



Parameter	Pr	mir-19a	mir-143	Combinated
AUC		0.74	0.75	0.81
95% CI (DeLong)		0.60-0.87	0.61-0.88	0.70-0.92
Sensitivity	Pr(+ D)	81.82%	78.79%	81.82%
Specificity	Pr(-~D)	56.00%	64.00%	56.00%
Positive predictive value	Pr(D +)	71.05%	74.29%	71.05%
Negative predictive value	Pr(~D -)	70.00%	69.57%	70.00%
Correctly classified		70.69%	72.41%	70.69%

Model	crude OR(95%CI)	adj. OR(95%CI)	P(LR-test)
miR-19a (high vs. low)	5.73 (1.75,18.75)	7.6 (1.97,29.38)	0.001
miR-143 (high vs. low)	6.07 (1.77,20.82)	12.54 (2.77,56.83)	< 0.001
Comb_mir19a (high vs. low)	5.73 (1.75,18.75)	3.55 (0.77,16.31)	0.097
Comb_mir143 (high vs. Low)	6.07 (1.77,20.82)	6.99 (1.31,37.24)	0.018

Fig. 3. Serum miRNAs associated with IMID severity. A) Normalised relative quantities (NRQs) of statistically significant miRNAs in serum samples from mild IMID patients compared with severe IMID patients. Results were analysed by a logistic regression model adjusted by age and sex. Data are presented as box-plots *P = 0.05. B) Receiver Operating Characteristic (ROC) curve analyses performed to assess the diagnostic value of circulating miR19a and miR-143 and the combination of the two miRNAs to discriminate between mild and severe disease. The table shows areas under the curve (AUC), percentages of correctly predicted cases, 95% confidence intervals (95% CIs), sensitivities, and specificities of the analyses. Results of the models are given as crude odds ratios (crude OR, 95% CI) and odds ratios adjusted by sex and age (adj. OR, 95% CI).

we have not observed significant differences in the relative expression of miR-21 and miR-126 in the IMIDs patients studied. This could be due to the combination in our analysis of several IMIDs as a whole group or to the comparison of all of them with controls. In this regard, previous studies have reported discordant results in these two miRNAs in IMIDs, with reports of decreased or increased expression depending of the specific disease or type of sample analysed (Supplementary Table 1).

Although enough reports in the literature describe the comparative profile of circulating miRNA levels between patients with individual autoimmune diseases and healthy donors, limited information has been published about miRNA profiles associated with specific phenotypes in patients with IMID, especially regarding disease severity. In this regard, we have described that two miRNAs (miR-19a and miR-143) belonging to cluster A show increased levels in patients with severe disease compared to those with mild disease. It is intriguing that the miRNAs that differentiate patients from healthy donors (miR-19b and miR26b) are located in a different cluster than those that differentiate severe and mild disease. This suggests that the pathways involved in the development of IMIDs can be different from those regulating their severity.

Regarding disease severity, our results showed an association between high levels of circulating miR-19a and miR-143 and patients with higher inflammatory status. Some targets of these miRNAs are proinflammatory molecules, such as TNFα [47,48], TLR2 [49] and molecules involved in MAPK signalling pathway [50], suggesting that miR-19a and miR-143 could be involved in negative feed-back loops. This may explain why miR-19a has been also associated with relapses of active psoriasis and with other IMIDs not assessed in this study such as multiple sclerosis, and inflammatory bowel disease [51–53].

Regarding miR-19a and the other members of cluster A1 (miR-101, miR-23a and miR-451), some of their targets also play anti-inflammatory or regulatory roles. These targets include the JAK/STAT pathway inhibitors SOCS1 and SOCS3 [54,55], the NFκB pathway inhibitors IKKa (also known as CHUK) and IKBKB [56,57], phosphatases like PTEN, which negatively regulates lymphocyte activation [58,59] and DUSP1 [60,61] and the TGFβ related transcription factor SMAD4 [62]. Therefore, in some IMIDs in which the regulation of TNF or TLR production or signalling is altered, the up-regulation of miR-19a and some other members of its cluster can result in perpetuation of disease with more severe forms due to down-regulation of anti-inflammatory pathways.

This research, however, is subject to some limitations. On one hand, cytokines have been described to modulate the expression of miRNAs and, on the other hand, miRNAs can modulate the expression of cytokines [63], thus it is difficult to determine about the causality of miRNAs in IMIDs. To evaluate whether altered expression of miRNAs are the cause or consequence of an IMID, it would be needed longitudinal studies in IMIDs patients, especially with samples and clinical data before and after treatment. This approach would provide valuable data on the contribution of our proposed miRNA signatures in the pathogenesis and their roles as biomarkers.

To conclude, it is possible that miR-19b and miR-26b could be used in the next future as promising diagnostic strategies for the prediction of development of IMIDs. In addition, the combination of miR-19a and miR-143 could serve as a biomarker to detect those patients with more severe disease who need early treatment. Altogether they may serve as a predictive signature for the future use of novel therapeutic targets for a successful treatment of IMIDs.

Declaration of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2020.102472.

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Author contributions

Conceptualization: FSM, IGA, MM; Data curation: RMH, HdlF, AL; Formal analysis: RMH, HdlF, AL, MSN, ASG; Funding acquisition: FSM, IGA, MM; Investigation: FSM, IGA, MM, RMH, HdlF, AL, MSN; Methodology: RMH, HdlF, AL; Project administration: FSM, IGA, MM; Resources: FSM, IGA, MM, RMH, HdlF, AL, MSN, ARL, ASS, RGV, AMO, ED, MLV, PC, PRJ, ASG; Supervision: FSM, IGA, MM, RMH, HdlF, AL, MSN; Validation: RMH, HdlF, AL; Visualization: FSM, IGA, MM, RMH, HdlF, AL, MSN; Roles/Writing - original draft: FSM, IGA, MM, RMH, HdlF, AL, MSN; Writing - review & editing: FSM, IGA, MM, RMH, HdlF, AL, MSN.

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