CONCISE REPORT

Galectin-9 is an easy to measure biomarker for the interferon signature in systemic lupus erythematosus and antiphospholipid syndrome

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ABSTRACT

Objective The interferon (IFN) signature is related to disease activity and vascular disease in systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) and represents a promising therapeutic target. Quantification of the IFN signature is currently performed by gene expression analysis, limiting its current applicability in clinical practice. Therefore, the objective of this study was to establish an easy to measure biomarker for the IFN signature.

Methods Serum levels of galectin-9, CXCL-10 (IP-10) and tumour necrosis factor receptor type II (TNF-RII) were measured in patients with SLE, SLE+APS and primary APS (PAPS) and healthy controls (n=148) after an initial screening of serum analytes in a smaller cohort (n=43). Analytes were correlated to measures of disease activity and the IFN signature. The performance of galectin-9, CXCL-10 and TNF-RII as biomarkers to detect the IFN signature was assessed by receiver operating characteristic curves.

Results Galectin-9, CXCL-10 and TNF-RII were elevated in patients with SLE, SLE+APS and PAPS ($p<0.05$) and correlated with disease activity and tissue factor expression. Galectin-9 correlated stronger than CXCL-10 or TNF-RII with the IFN score ($r=0.70$, $p<0.001$) and was superior to CXCL-10 or TNF-RII in detecting the IFN signature (area under the curve (AUC) 0.86). Importantly, in patients with SLE(±APS), galectin-9 was also superior to anti-dsDNA antibody (AUC 0.70), or complement C3 (AUC 0.70) and C4 (AUC 0.78) levels in detecting the IFN signature.

Conclusion Galectin-9 is a novel, easy to measure hence clinically applicable biomarker to detect the IFN signature in patients with systemic autoimmune diseases such as SLE and APS.

Key messages

What is already known about this subject?
- The interferon signature is an imporant biomarker for disease activity and vascular disease in systemic lupus erythematosus and antiphospholipid syndrome.
- The interferon signature is assessed by gene-expression analysis which hampers its implication in clinical practice.

What does this study add?
- Galectin-9 correlates with disease activity and is a robust and easy to measure serum biomarker to detect the interferon signature in systemic lupus erythematosus and antiphospholipid syndrome.
- Galectin-9 outperforms CXCL-10 or traditional markers of disease activity to detect the interferon signature in systemic lupus erythematosus and antiphospholipid syndrome.

How might this impact on clinical practice or futue developments?
- Serum levels of galectin-9 may aid clinical decision making in steering anti-interferon targeted therapies in patients with systemic lupus erythematosus and antiphospholipid syndrome.

Systemic lupus erythematosus (SLE) is a chronic relapsing autoimmune disease in which immune complexes of autoantibodies are deposited in tissues inducing tissue damage. Approximately 20% of patients with SLE have antiphospholipid syndrome (APS), defined as the persistent presence of antiphospholipid (aPL) antibodies in patients who experienced at least one thrombotic or predefined obstetric complication. APS also affects the patients without an underlying disease and is then termed primary APS (PAPS). The pathogenesis of SLE and APS is partly overlapping including shared genetic risk loci and perturbations in both the innate and adaptive immune system, although both conditions are only rarely studied together.[1]

Transcriptomic studies in SLE and APS have revealed a markedly increased expression of interferon (IFN) inducible genes, known as the IFN signature which is present in ~75% of patients with SLE (±APS) and ~50% of patients with PAPS.[2–5] In SLE, the IFN signature is associated with elevated autoantibody levels, disease activity, future flares and congenital heart block.[3 4 6 7] In (P)APS, the IFN signature has only recently been reported and has been linked to endothelial progenitor cell dysfunction and increases in proinflammatory monocytes.[2 5]

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The IFN signature is a promising therapeutic target. Anifrolumab, a monoclonal antibody against IFNAR, the receptor for type I IFN, showed clinical efficacy in a phase IIb clinical trial in SLE, particularly in those patients with an IFN signature.[8] The detection of the IFN signature may therefore soon be used to guide treatment decisions in SLE and possibly other autoimmune diseases.

The IFN signature is measured by gene expression analysis. Therefore, easier to measure biomarkers for IFN signature detection are urgently needed. Measuring IFNα in peripheral blood seems the most straightforward method. However, IFNα is difficult to detect by standard techniques[9] and the 12 different IFNα proteins cannot be measured by a single assay. In addition, other type I IFNs (including IFNβ) as well as type II and III IFN (IFNγ and IFNλ) contribute to the IFN signature.[10] Therefore, surrogate markers for the IFN signature were searched for Siglec-1 and CD64 expression on monocytes, and serum levels of CXCL-10 correlate with the IFN signature in SLE and serve as alternatives to detect the IFN signature in SLE.[6 11–13]

Here, using an identification and replication approach we identified that galectin-9, CXCL-10 and tumour necrosis factor receptor type II (TNF-RII) correlate with the IFN signature and disease activity in patients with SLE and APS. Importantly, galectin-9 outperformed CXCL-10 or traditional markers of disease activity as an easy measurable biomarker to detect the IFN signature both in SLE and APS.

METHODS

Blood was drawn from patients with SLE, SLE+APS, PAPS and healthy controls (HC, table 1). Patients with PAPS did not meet the American College of Rheumatology criteria for SLE.[14] The medical ethical committee of the UMC Utrecht approved the study and written informed consent was obtained.

Clinical and laboratory assessments

Serum analytes were measured as described in the online supplementary methods[15] in an initial identification cohort (n=43), followed by a replication step (n=148). For serum analytes, elevated levels were defined as 2 SDs above the mean of HC. Anti-dsDNA antibodies and complement levels were determined by EliA or nephelometry. Details on the assessment of the IFN signature, tissue factor (TF) expression and mRNA expression of galectin-9 in dendritic cells (DC) are provided in the online supplementary methods.

Statistics

All tests were conducted two sided at an α level of 0.05. Statistical tests used are provided in the figure legends.

Table 1 Clinical characteristics

	HC (n=27)	SLE (n=50)	SLE+APS (n=40)	PAPS (n=29)
Female (%)	93	96	95	97
Age	43 (34–50)	40 (28–48)	45 (37–53)	40 (33–50)
Disease manifestations				
SLEDAI		4 (2–6)	4 (1–5)	–
Malar rash (%)		66	55	0
Discoid rash (%)		22	18	0
Photosensitivity (%)		42	53	0
Oral ulcers (%)		36	35	0
Arthritis (%)		70	65	0
Serositis (%)		28	20	0
Lupus nephritis (%)		60	45	0
Neurologic disorder (%)		4	18	10
Haematological disorder (%)		60	85	35
Arterial thrombosis (%)		10	43	59
Venous thrombosis (%)		4	60	38
Obstetrical morbidity (%)		6	23	31
Current drug use				
Hydroxychloroquine (%)		80	55	21
Prednisone (%)		64	45	0
Azathioprine (%)		34	33	0
Oral anticoagulant (%)		2	75	62
Aspirin (%)		22	28	48
Serology				
C3 (g/L)		0.88 (0.69–1.03)	0.79 (0.68–0.92)	–
C4 (g/L)		0.16 (0.13–0.20)	0.15 (0.11–0.22)	–
a-dsDNA (IU/mL)		27 (6–114)	14 (5–58)	–
Lupus anticoagulant (%)		13	65	82
IgG aCL (%)		15	78	86
IgM aCL (%)		11	15	38
IgG aβ2GPI (%)		7	26	35
IgM aβ2GPI (%)		7	5	10

Medians with IQR or percentage of total.
aβ2GPI, anti-β2 glycoprotein I antibodies; aCL, anticardiolipin antibodies; a-dsDNA, anti-double stranded DNA antibodies; APS, antiphospholipid syndrome; HC, healthy control; PAPS, primary APS; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

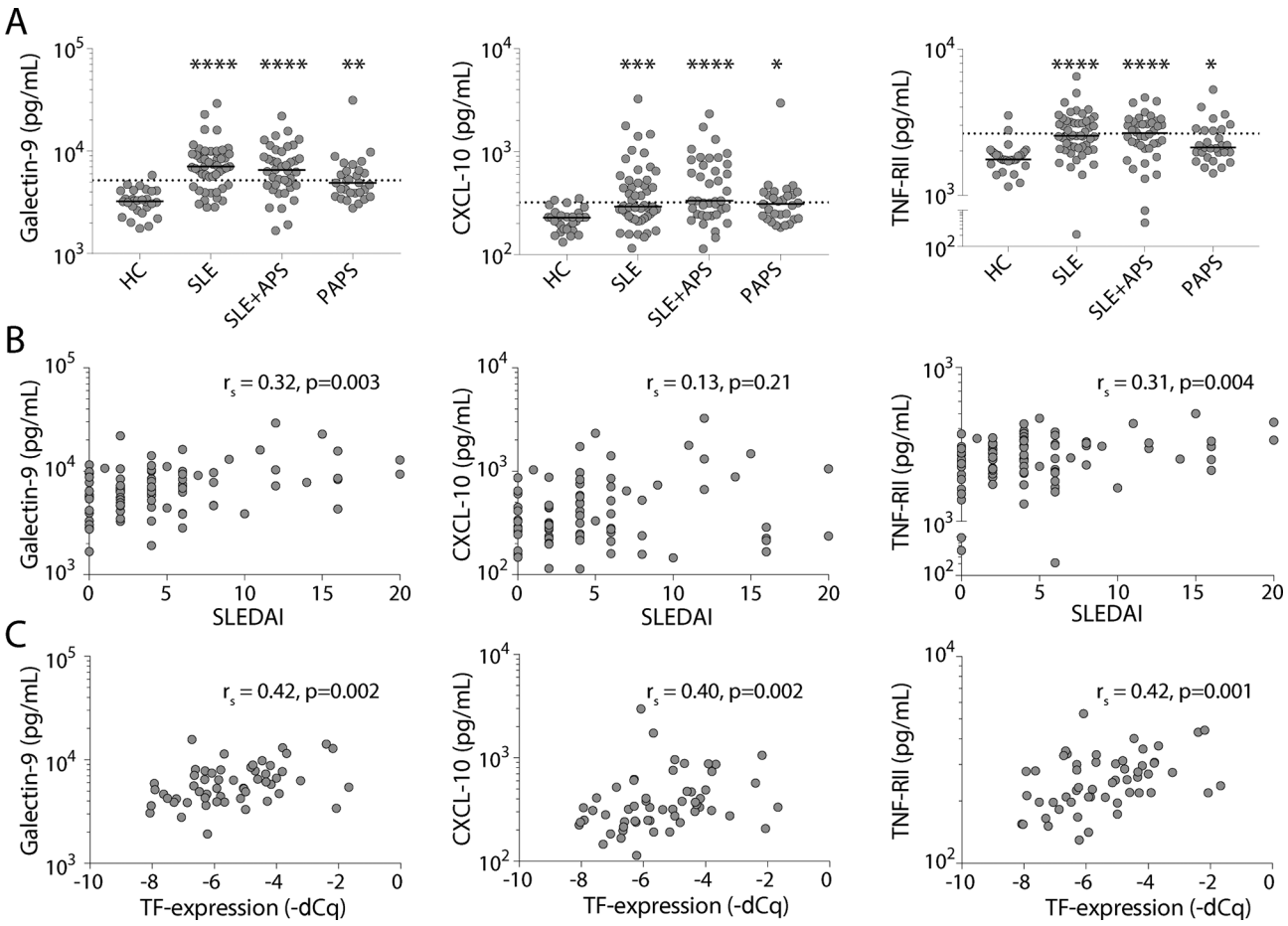


Figure 1 Galectin-9 is a biomarker for disease activity and tissue factor expression in SLE and APS. (A) Galectin-9, CXCL-10 and TNF-RII were measured by Luminex in sera of patients with SLE, SLE+APS and PAPS (Statistics: Kruskal-Wallis with post hoc Dunn's). Dotted line represents the mean of HC+2 SD. (B) Correlation of galectin-9, CXCL-10 and TNF-RII with disease activity in patients with SLE and SLE+APS. (C) Correlation of galectin-9, CXCL-10 and TNF-RII tissue factor (TF) expression in monocytes in patients with SLE+APS and PAPS as assessed by qPCR (-dCq, delta quantification cycle). Statistics: (A) Kruskal-Wallis followed by post hoc Dunn's. (B, C) Spearman correlation coefficients. APS, antiphospholipid syndrome; HC, healthy control; PAPS, primary APS; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index; TNF-RII, tumour necrosis factor receptor type II.

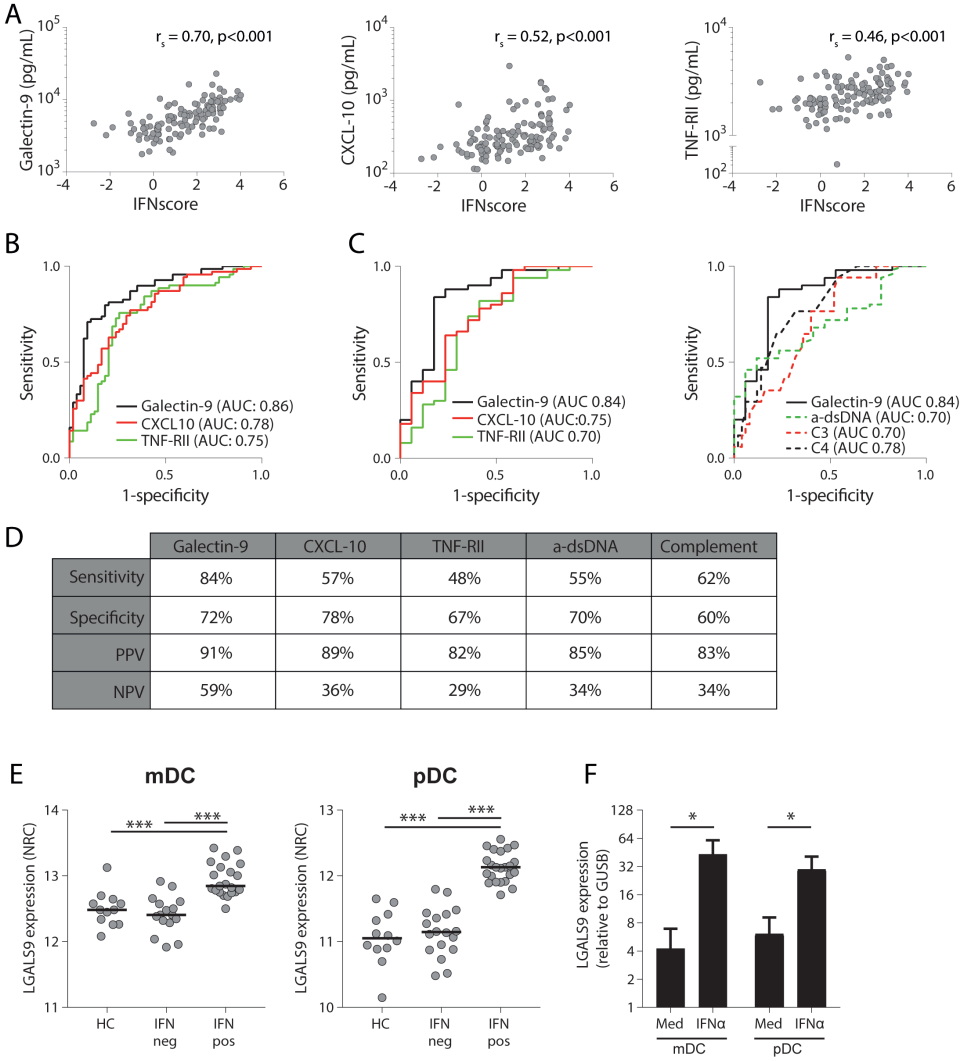
RESULTS

Galectin-9, CXCL-10 and TNF-RII are biomarkers of disease activity in SLE and APS

In an initial screening of 22 serum analytes, galectin-9 (r=0.81, p<0.001), CXCL-10 (r=0.72, p<0.001) and TNF-RII (r=0.42, p=0.007) correlated significantly with the IFN score in patients with SLE, SLE+APS and PAPS (n=43, online supplementary table and figure 1). We next assessed these analytes in a larger replication step (n=148). Galectin-9, CXCL-10 and TNF-RII levels were significantly elevated in patients with SLE, SLE+APS and PAPS as compared with HC (figure 1A). Elevated levels of galectin-9 were present in 74%, 68% and 41% of patients with SLE, SLE+APS and PAPS, respectively, compared with 46%, 55% and 45% for CXCL-10% and 46%, 50% and 41% for TNF-RII. All three analytes correlated with anti-dsDNA antibodies and complement levels in patients with SLE (online supplementary figure 2). Furthermore, galectin-9 and TNF-RII, not CXCL-10, correlated with disease activity as assessed by SLE Disease Activity Index (figure 1B). In patients with APS, the increased expression of TF by monocytes is induced by aPL antibodies and reflects a prothrombotic phenotype,[1] and we found significant correlations between galectin-9, CXCL-10 and TNF-RII and the expression of TF by monocytes in patients with APS (figure 1C).

Galectin-9 is a biomarker to detect the IFN signature in SLE and APS

A superior correlation of galectin-9 with the IFN score (r=0.70, p<0.001) as compared with CXCL-10 (r=0.52, p<0.001) and TNF-RII (r=0.46, p<0.001) was found (figure 2A). Receiver operating characteristic curve analysis revealed the highest area under the curve (AUC, 0.86) for galectin-9, followed by CXCL-10 (0.78) and TNF-RII (0.75) for the detection of the IFN signature (figure 2B) among patients with SLE or APS. When focusing only on patients with SLE(±APS), galectin-9 (AUC 0.84) remained a superior biomarker to detect the IFN signature compared with CXCL-10 (AUC 0.75) or TNF-RII (AUC 0.70) or traditional markers of SLE disease activity such as anti-dsDNA antibodies (AUC 0.70) or complement levels (C3: AUC 0.70, C4: AUC 0.78) (figure 2C). Elevated levels of galectin-9 had a sensitivity of 84% and a positive predictive value of 91% of detecting the IFN signature among patients with SLE(±APS)



	Galectin-9	CXCL-10	TNF-RII	a-dsDNA	Complement
Sensitivity	84%	57%	48%	55%	62%
Specificity	72%	78%	67%	70%	60%
PPV	91%	89%	82%	85%	83%
NPV	59%	36%	29%	34%	34%

Figure 2 In SLE and APS, galectin-9 outperforms CXCL-10 and traditional markers for disease activity as biomarker for the IFN signature and is upregulated in dendritic cells. (A) Correlation of galectin-9, CXCL-10 and TNF-RII with the IFN score. (B) ROC curve analysis of galectin-9, CXCL-10 and TNF-RII in the detection of the IFN signature in patients with SLE, SLE+APS and PAPS. (C) ROC curve analysis of galactin-9, CXCL-10 and TNF-RII as compared with traditional markers of SLE disease activity in detecting the IFN signature among patients with SLE±APS. (D) Test characteristics of elevated galectin-9, CXCL-10, TNF-RII, anti-dsDNA antibodies or reduced complement levels to detect the IFN signature in patients with SLE(±APS). (E) Expression of galectin-9 coding gene LGALS9 in myeloid and plasmacytoid DCs as assessed by RNAseq in patients with SLE and APS stratified by IFN status. (F) Expression of LGALS9 assessed by qPCR after in vitro stimulation of DCs with IFNα for 3 hours (n=3/group). Statistics: (A) Spearman correlation coefficients. (E) ANOVA with post hoc Tukey's or Student's t-test. (F) Student's t-test. ANOVA, analysis of variance; APS, antiphospholipid syndrome; AUC, area under the curve; HC, healthy control; IFN, interferon; mDC, myeloid dendritic cells; NPV, negative predictive value; PAPS, primary APS; pDC, plasmacytoid dendritic cells; PPV, positive predictive value; ROC, receiver operating characteristic; SLE, systemic lupus erythematosus; TNF-RII, tumour necrosis factor receptor type II.

(figure 2D). In multivariate linear regression analysis galectin-9 was an independent predictor for the IFN score, and remained significant after correction for anti-dsDNA antibodies, complement levels and disease activity.

Increased expression of galectin-9 in DCs of IFN-positive patients with SLE and APS

Galectin-9 is a β-galactoside-binding lectin, encoded by the gene LGALS9 which is highly expressed in DCs.[16] Therefore, we explored the expression of LGALS9 in myeloid and plasmacytoid DCs (mDC/pDC) in SLE and APS. LGALS9 expression was markedly higher in circulating DCs of patients with SLE and APS with an IFN signature and IFNα increased LGALS9 expression in vitro in mDCs and pDCs (figure 2E,F).

DISCUSSION

Here we report that galectin-9, CXCL-10 and TNF-RII are elevated and associated with disease activity in SLE and APS. Notably, we identified galectin-9 as a robust and easy to measure biomarker to detect the IFN signature, outperforming traditional biomarkers for the IFN signature such as CXCL-10.

Measuring the IFN signature in patients with SLE might in the future be used in clinical decision-making.[8] The necessity to quantify gene expression and the lack of a uniformly accepted gene set or scoring system hamper its implementation in clinical practice. Furthermore, serum biomarkers are more easily standardised and are less laborious as compared with previously proposed biomarkers for the IFN signature that are assessed by flow cytometry.[12 13] In this respect, galectin-9 is a promising

stable biomarker, which is superior to CXCL-10 in the detection of the IFN signature, both in SLE and APS, and can furthermore be reliably detected in serum samples stored for long periods.[17]

Besides serving as a biomarker, galectin-9 may play a role in the pathogenesis of SLE and APS. Galectin-9 induces the maturation of DCs by increasing the expression of HLA-DR and costimulatory molecules and galectin-9 produced by DCs activates T cells.[16] Galectin-9 expression was markedly increased in DCs of IFN-positive patients with SLE and APS and may therefore drive the unabated activation of DCs seen in these patients. Furthermore, in the pristane-induced SLE mouse model, knockout of LGALS9 results in reduced nephritis and arthritis,[18] supporting a pathogenic role for galectin-9 in the pathogenesis of SLE. Besides IFNα, galectin-9 may also be induced by other proinflammatory mediators implicated in the pathophysiology of autoimmune diseases,[19] hence the applicability of galectin-9 as a biomarker for immunopathology in other diseases warrants further study.

The treatment of SLE and APS has remained largely unchanged for decades. The revolution in the treatment of other rheumatic conditions, most notably rheumatoid arthritis, in the last decades has not been paralleled in SLE and APS in which few clinical trials have met their primary outcome. A major reason for the failure of these trials is the molecular heterogeneity of these diseases. Consequently, for SLE and APS there is an urgent need for a more personalised treatment approach, guided by the molecular phenotype rather than clinical diagnosis. Treatment of patients on the basis of the presence of an IFN signature could represent such a molecular phenotype, supported by the results from the anifrolumab trial.[8] Galectin-9 may serve as a robust and easy to measure biomarker to detect the IFN signature in patients with SLE and APS, as well as other (systemic) autoimmune diseases.

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