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DOI:

[10.1016/j.jaut.2019.102340](https://doi.org/10.1016/j.jaut.2019.102340)

*Document Version*

Publisher's PDF, also known as Version of record

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*Citation for published version (APA):*

Enocsson, H., Wirestam, L., Dahle, C., Padyukov, L., Jönsen, A., Urowitz, M. B., Gladman, D. D., Romero-Diaz, J., Bae, S. C., Fortin, P. R., Sanchez-Guerrero, J., Clarke, A. E., Bernatsky, S., Gordon, C., Hanly, J. G., Wallace, D. J., Isenberg, D. A., Rahman, A., Merrill, J. T., ... Sjöwall, C. (2020). Soluble urokinase plasminogen activator receptor (suPAR) levels predict damage accrual in patients with recent-onset systemic lupus erythematosus. *Journal of Autoimmunity*, 106, Article 102340. <https://doi.org/10.1016/j.jaut.2019.102340>

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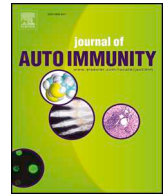
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## Soluble urokinase plasminogen activator receptor (suPAR) levels predict damage accrual in patients with recent-onset systemic lupus erythematosus



Helena Enocsson<sup>a,\*,1</sup>, Lina Wirestam<sup>a,1</sup>, Charlotte Dahle<sup>b</sup>, Leonid Padyukov<sup>c</sup>, Andreas Jönsen<sup>d</sup>, Murray B. Urowitz<sup>e</sup>, Dafna D. Gladman<sup>e</sup>, Juanita Romero-Diaz<sup>f</sup>, Sang-Cheol Bae<sup>g</sup>, Paul R. Fortin<sup>h</sup>, Jorge Sanchez-Guerrero<sup>e</sup>, Ann E. Clarke<sup>i</sup>, Sasha Bernatsky<sup>j</sup>, Caroline Gordon<sup>k</sup>, John G. Hanly<sup>l</sup>, Daniel J. Wallace<sup>m</sup>, David A. Isenberg<sup>n</sup>, Anisur Rahman<sup>n</sup>, Joan T. Merrill<sup>o</sup>, Ellen Ginzler<sup>p</sup>, Graciela S. Alarcón<sup>q</sup>, W. Winn Chatham<sup>q</sup>, Michelle Petri<sup>f</sup>, Munther Khamashta<sup>s</sup>, Cynthia Aranow<sup>t</sup>, Meggan Mackay<sup>t</sup>, Mary Anne Dooley<sup>u</sup>, Susan Manzi<sup>v</sup>, Rosalind Ramsey-Goldman<sup>w</sup>, Ola Nived<sup>d</sup>, Kristjan Steinsson<sup>x</sup>, Asad A. Zoma<sup>y</sup>, Guillermo Ruiz-Irastorza<sup>z</sup>, S. Sam Lim<sup>aa</sup>, Kenneth C. Kalunian<sup>ab</sup>, Murat Inanc<sup>ac</sup>, Ronald F. van Vollenhoven<sup>ad,ae,af</sup>, Manuel Ramos-Casals<sup>ag</sup>, Diane L. Kamen<sup>ah</sup>, Søren Jacobsen<sup>ai</sup>, Christine A. Peschken<sup>aj</sup>, Anca Askanase<sup>ak</sup>, Thomas Stoll<sup>al</sup>, Ian N. Bruce<sup>am</sup>, Jonas Wetterö<sup>a</sup>, Christopher Sjöwall<sup>a</sup>

<sup>a</sup> Rheumatology/Division of Neuro and Inflammation Sciences, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

<sup>b</sup> Department of Clinical Immunology and Transfusion Medicine and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

<sup>c</sup> Department of Medicine, Division of Rheumatology, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

<sup>d</sup> Department of Clinical Sciences Lund, Section of Rheumatology, Lund University, Lund, Sweden

<sup>e</sup> Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital and University of Toronto, Toronto, Ontario, Canada

<sup>f</sup> Instituto Nacional de Ciencias Médicas y Nutrición, Mexico City, Mexico

<sup>g</sup> Department of Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul, South Korea

<sup>h</sup> Division of Rheumatology, CHU de Québec - Université Laval, Quebec City, Canada

<sup>i</sup> Division of Rheumatology, Cumming School of Medicine University of Calgary, Calgary, Alberta, Canada

<sup>j</sup> Division of Rheumatology, Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada

<sup>k</sup> Rheumatology Research Group, Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK

<sup>l</sup> Division of Rheumatology, Department of Medicine and Department of Pathology, Queen Elizabeth II Health Sciences Centre and Dalhousie University, Halifax, Nova Scotia, Canada

<sup>m</sup> Cedars-Sinai/David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

<sup>n</sup> Centre for Rheumatology Research, University College, London, UK

<sup>o</sup> Department of Clinical Pharmacology, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

<sup>p</sup> Department of Medicine, SUNY Downstate Medical Center, Brooklyn, NY, USA

<sup>q</sup> Department of Medicine, Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, AL, USA

<sup>r</sup> Department of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

<sup>s</sup> Lupus Research Unit, The Rayne Institute, St Thomas' Hospital, King's College London School of Medicine, London, UK

<sup>t</sup> Feinstein Institute for Medical Research, Manhasset, NY, USA

<sup>u</sup> Division of Rheumatology and Immunology, Department of Medicine, University of North Carolina, Chapel Hill, NC, USA

<sup>v</sup> Autoimmunity Institute, Allegheny Health Network, Pittsburgh, PA, USA

<sup>w</sup> Northwestern University and Feinberg School of Medicine, Chicago, IL, USA

<sup>x</sup> Department of Rheumatology, Center for Rheumatology Research Fossvogur, Landspítali University Hospital, Reykjavik, Iceland

<sup>y</sup> Lanarkshire Centre for Rheumatology, Hairmyres Hospital, East Kilbride, Scotland, UK

<sup>z</sup> Autoimmune Disease Unit, Department of Internal Medicine, Hospital Universitario Cruces, BioCruces Health Research Institute, University of the Basque Country, Barakaldo, Spain

<sup>aa</sup> Division of Rheumatology, Emory University School of Medicine, Atlanta, GA, USA

<sup>ab</sup> UCSD School of Medicine, La Jolla, CA, USA

<sup>ac</sup> Division of Rheumatology, Department of Internal Medicine, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

<sup>ad</sup> Amsterdam University Medical Centres, University of Amsterdam, Amsterdam, Netherlands

<sup>ae</sup> Free University (VU) Amsterdam, Amsterdam, Netherlands

<sup>af</sup> Amsterdam Rheumatology and Immunology Center, Amsterdam, Netherlands

<sup>ag</sup> Josep Font Autoimmune Diseases Laboratory, IDIBAPS, Department of Autoimmune Diseases, Hospital Clínic, Barcelona, Spain

<sup>ah</sup> Division of Rheumatology, Medical University of South Carolina, Charleston, SC, USA

\* Corresponding author. AIR, Department of Clinical and Experimental Medicine, Campus US, Linköping University, SE-581 85 Linköping, Sweden.  
E-mail address: [helena.enocsson@liu.se](mailto:helena.enocsson@liu.se) (H. Enocsson).

<sup>1</sup> Contributed equally to this study.

<https://doi.org/10.1016/j.jaut.2019.102340>

Available online 17 October 2019

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<sup>a1</sup> Copenhagen Lupus and Vasculitis Clinic, Centre for Rheumatology and Spine Diseases, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

<sup>a2</sup> Department of Medicine and Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

<sup>ak</sup> Division of Rheumatology, Columbia University Medical Center, New York, USA

<sup>al</sup> Department of Rheumatology, Kantonsspital, Schaffhausen, Switzerland

<sup>am</sup> Arthritis Research UK Centre for Epidemiology, Centre for Musculoskeletal Research, Faculty of Biology, Medicine and Health, The University of Manchester and NIHR Manchester Biomedical Research Centre, Manchester University Hospital NHS Foundation Trust, Manchester Academic Health Science Center, Manchester, UK

## ARTICLE INFO

### Keywords:

Biomarker

SLE

Organ damage

Prognosis

Outcome

## ABSTRACT

**Objective:** The soluble urokinase plasminogen activator receptor (suPAR) has potential as a prognosis and severity biomarker in several inflammatory and infectious diseases. In a previous cross-sectional study, suPAR levels were shown to reflect damage accrual in cases of systemic lupus erythematosus (SLE). Herein, we evaluated suPAR as a predictor of future organ damage in recent-onset SLE.

**Methods:** Included were 344 patients from the Systemic Lupus International Collaborating Clinics (SLICC) Inception Cohort who met the 1997 American College of Rheumatology classification criteria with 5-years of follow-up data available. Baseline sera from patients and age- and sex-matched controls were assayed for suPAR. Organ damage was assessed annually using the SLICC/ACR damage index (SDI).

**Results:** The levels of suPAR were higher in patients who accrued damage, particularly those with  $SDI \geq 2$  at 5 years ( $N = 32$ , 46.8% increase,  $p = 0.004$ ), as compared to patients without damage. Logistic regression analysis revealed a significant impact of suPAR on SDI outcome ( $SDI \geq 2$ ; OR = 1.14; 95% CI 1.03–1.26), also after adjustment for confounding factors. In an optimized logistic regression to predict damage, suPAR persisted as a predictor, together with baseline disease activity (SLEDAI-2K), age, and non-Caucasian ethnicity (model AUC = 0.77). Dissecting SDI into organ systems revealed higher suPAR levels in patients who developed musculoskeletal damage ( $SDI \geq 1$ ;  $p = 0.007$ ).

**Conclusion:** Prognostic biomarkers identify patients who are at risk of acquiring early damage and therefore need careful observation and targeted treatment strategies. Overall, suPAR constitutes an interesting biomarker for patient stratification and for identifying SLE patients who are at risk of acquiring organ damage during the first 5 years of disease.

## 1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with unpredictable disease course, diverse manifestations, and fluctuating disease activity. A deficiency in the system for disposing of dying cells, the production of antinuclear autoantibodies (ANA), neutrophil extracellular trap (NET) formation, activation of type I interferon (IFN) signalling, and subsequent tissue damage appear to be important in the pathogenesis of SLE, which is often manifested as rash, arthritis, and nephritis. In many patients, the persistent inflammation and drug-related side-effects eventually cause permanent organ damage, which is strongly linked to mortality [1–3].

Apart from a few useful laboratory measures to assess SLE disease activity, i.e., specific autoantibodies and complement proteins, biomarkers that indicate the prognosis and the risk of acquiring damage over time are sparse, possibly due to the heterogeneity of the disease. Furthermore, it is challenging to distinguish symptoms caused by active disease from those that arise following permanent organ damage [4]. C-reactive protein (CRP), which is the standard biomarker of inflammation, gives limited information about SLE disease activity, possibly due to negative regulation by IFN- $\alpha$ , in combination with a CRP polymorphism that is more frequently found in patients with SLE [5,6]. Nevertheless, an association between CRP levels and future damage accrual in patients with SLE has been suggested [7,8]. Other biomarkers have not, in a convincing way, been associated with prediction of organ damage. ANAs have yielded ambiguous results [1,9] and osteopontin was of limited value [10].

The membrane-bound urokinase plasminogen activator receptor (uPAR; CD87) is a multi-ligand receptor that interacts with urokinase-type plasminogen (uPA; also known as urokinase), thereby regulating fibrinolysis and tissue remodelling [11]. Furthermore, uPAR has affinity for integrins and vitronectin, through which it coordinates cell migration and adhesion to the extracellular matrix [12]. Of interest for the pathogenesis of SLE, uPAR appears to regulate the phagocytosis of apoptotic cells, i.e., efferocytosis [13–15]. Proteolytic cleavage of uPAR generates the soluble form, suPAR [11]. Increased levels of suPAR have

been implicated in the development of renal dysfunction, possibly through a direct effect on podocyte behaviour [16]. Studies on uPAR expression suggest that it is present on endothelial cells, smooth muscle cells, and various immune cells [17–20] and suPAR levels are found to correlate with leukocyte count [8,21,22].

The potential of suPAR as a biomarker that can be used in routine clinical practice is strengthened by: 1) its *in vitro* stability in serum/plasma over time and during repeated freeze-thaw cycles [23]; 2) its insensitivity to diurnal variations [21]; and 3) the absence of polymorphisms in the uPAR gene promoter that affect the baseline suPAR levels [24]. Furthermore, suPAR is under evaluation as a marker of decreased survival in acute medical settings [25].

Given the linkages between suPAR levels and the severity of inflammatory diseases [16,26–29], we previously investigated the potential of suPAR as a biomarker in SLE [8,30]. We found that suPAR, adjusted for leukocyte count, was increased in SLE and that levels reflected established organ damage [8]. In addition, significantly higher levels of suPAR occurred in patients with a higher score on the Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index (SDI) in the 2–5 years following their inclusion, as compared with patients without an increase in the SDI score [30], thereby suggesting a predictive value of suPAR.

The aim of this study was to investigate if suPAR predicts future organ damage accrual in a longitudinal, international, inception cohort of recent-onset SLE cases, unbiased from previous organ damage.

## 2. Materials and methods

### 2.1. The SLICC inception cohort

The Systemic Lupus International Collaborating Clinics (SLICC) Inception Cohort ( $N = 1848$ ) was recruited at 33 centres in 11 countries in North America, Europe, and Asia during the period 2000–2011, as previously described [1,31]. All the clinical data were submitted to the coordinating centre at the University of Toronto, and the patients were reviewed annually. Laboratory tests for evaluating disease activity

and the recording of organ damage parameters were performed locally. During the first 5 years of follow-up, 3.0% of the patients were deceased, 14.9% were lost to follow-up for other reasons, and 24.8% had not yet reached 5 years of follow-up at the time of data extraction.

This study was approved by the SLICC data coordinating centre's institutional Research Ethics Board at the University Health Network (File#: 00-0279). At each of the 33 participating centres, Ethics Review Boards approved the SLICC Inception Cohort study.

## 2.2. Patients and controls

The study population (N = 344; Table 1) consisted of a sub-population from the SLICC Inception Cohort that had 5 years of annual follow-up data, no organ damage at baseline, and baseline serum sample availability (Fig. 1). All the cases were classified according to the ACR 1997 (ACR-97) criteria [32] and enrolled within 15 months (median, 5 months; range, 0–15 months) of SLE diagnosis. Use of corticosteroids, antimalarials and/or immunosuppressants (e.g. cyclophosphamide, azathioprine, mycophenolate mofetil, methotrexate or cyclosporine) by the time of enrolment was recorded. The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) [33] and SDI [34] scores were assessed at each annual visit. At baseline, peripheral venous blood was drawn from each individual. Sera were stored at  $-70^{\circ}\text{C}$  until analysed.

Sera from population-based controls (Table 1), matched 1:1 for sex and age, included in the *Swedish Epidemiological Investigation of Rheumatoid Arthritis* (EIRA) cohort served as controls for the suPAR analyses [35]. All the patients and controls provided written informed consent.

## 2.3. Clinical and laboratory data

SLEDAI-2K calculation was performed locally at each participating centre. A cumulative SLEDAI-2K score was calculated based on the addition of the SLEDAI-2K score at every yearly visit from inclusion (baseline) to year 5. The cumulative SLEDAI-2K score was therefore based on 6 visits and was available for 337 of the patients (98%). The level of creatinine, which was analysed at Linköping University hospital, Sweden, was used to calculate the estimated glomerular filtration rate (eGFR) according to the MDRD 4-Variable Equation [36], or the Bedside Schwartz equation for patients younger than 18 years (N = 8) [37]. In addition, all the baseline samples were analysed for ANA fine-specificities using addressable laser bead immunoassay (ALBIA) and the FIDIS™ Connective profile, Solonium software ver. 1.7.1.0 (Theradiag, Croissy-Beaubourg, France) in Linköping [38]. Complement (C3 and C4) levels were measured at the local centres (N = 308), or in Linköping if data were missing (N = 36). The erythrocyte sedimentation rate (ESR) and CRP measurements were performed at the local centres. Baseline CRP was available for 258 cases and results below 5 mg/L were given the same value (2.5 mg/L).

## 2.4. Baseline suPAR analysis

A clinically validated immunoassay (suPARnostic® AUTOFlex ELISA; ViroGates, Birkerød, Denmark) was used according to manufacturer's instructions. The concentration of suPAR in baseline serum was measured for all patients and controls. Serum and peroxidase-conjugated anti-suPAR were incubated in microwells pre-coated with anti-suPAR antibodies. After incubation, a tetramethylbenzidine substrate was added and the reaction was stopped by 2 N sulfuric acid. The optical density was detected at 450 nm (Sunrise plate reader, Magellan ver. 7.1 software, Tecan, Männedorf, Switzerland). Mean serum suPAR in blood donors from Linköping, Sweden (N = 100, 50% women) is 3.97 ng/mL [8]. A Danish study of 5538 individuals showed a mean serum concentration of suPAR of 3.51 ng/mL for men and 3.90 ng/mL for women [39].

## 2.5. Statistical analyses

Independent samples *t*-test and Pearson correlation analyses were performed. Due to the small subgroups, the Mann-Whitney *U* test (exact method) was used instead of the independent samples *t*-test when patients were distinguished based on damage in specific organ domains. When the Mann-Whitney *U* test is applied, an approximate 95% confidence interval (CI) is given (actual confidence level is stated). One-way ANOVA (with Hochberg's post-hoc test) was used to reveal differences in suPAR levels between ethnic groups and between patients grouped according to SDI. Binary logistic regression (enter or forward stepwise likelihood ratio method) was used to predict global or specific damage accumulation. Receiver operator characteristics (ROC) curve and calculations of the area under the curve (AUC) were based on predicted probabilities from the respective regression model. *P*-values < 0.05 were considered statistically significant. The statistical analyses were performed with the SPSS Statistics 23 (IBM, Armonk, NY, USA) or GraphPad Prism, ver. 8.0.1 (GraphPad Software, San Diego, CA) software packages.

## 3. Results

### 3.1. Cohort outcome

In total, 344 patients with SLE (mean age, 34.0 years; 91.6% women) were included. The majority (58.1%) were Caucasians. Of the 344 controls (mean age, 34.4 years; 91.6% women), 95.1% were

**Table 1**  
Clinicodemographic characteristics of the 344 patients with SLE and 344 matched population-based controls.

|  | Mean (range) or number (%) |              |
|--|----------------------------|--------------|
|  | SLE patients               | Controls     |
| Age at inclusion (years)                       | 34.0 (12–73)               | 34.4 (15–73) |
| Female sex                                     | 315 (91.6)                 | 315 (91.6)   |
| Ethnicity                                      |                            |              |
| Caucasian                                      | 200 (58.1)                 | 327 (95.1)   |
| Asian  | 64 (18.6)                  | 3 (0.9)      |
| African ancestry                               | 52 (15.1)                  | 4 (1.1)      |
| Other (Mixed, Native American, Hispanic)       | 28 (8.1)                   | 10 (2.9)     |
| SLEDAI-2K at baseline (score)                  | 5.0 (0–30)                 | N/A          |
| eGFR at baseline (mL/min/1.73 m <sup>2</sup> ) | 120 (6–473)                | N/A          |
| Abnormal eGFR (< 90)                           | 79 (23.0)                  | N/A          |
| Antimalarials                                  | 242 (70.3)                 | N/A          |
| Immunosuppressants                             | 122 (35.5)                 | N/A          |
| Corticosteroids                                | 222 (64.5)                 | N/A          |
| Corticosteroid dose at baseline (mg/day)       | 13.9 (0–90)                | N/A          |
| SLICC/ACR damage index (SDI ≥ 1) at 1 year     | 19 (5.5)                   | N/A          |
| SLICC/ACR damage index (SDI ≥ 1) at 2 years    | 36 (10.5)                  | N/A          |
| SLICC/ACR damage index (SDI ≥ 1) at 3 years    | 63 (18.3)                  | N/A          |
| SLICC/ACR damage index (SDI ≥ 1) at 4 years    | 79 (23.0)                  | N/A          |
| SLICC/ACR damage index (SDI ≥ 1) at 5 years    | 98 (28.5)                  | N/A          |
| Domains of damage (SDI ≥ 1) at 5 years         |                            |              |
| Ocular   | 27 (7.8)                   | N/A          |
| Musculoskeletal                                | 23 (6.7)                   | N/A          |
| Neuropsychiatric                               | 20 (5.8)                   | N/A          |
| Skin   | 19 (5.5)                   | N/A          |
| Pulmonary                                      | 7 (2.0)                    | N/A          |
| Renal  | 6 (1.7)                    | N/A          |
| Peripheral vascular                            | 6 (1.7)                    | N/A          |
| Cardiovascular                                 | 5 (1.5)                    | N/A          |
| Gastrointestinal                               | 5 (1.5)                    | N/A          |
| Malignancy                                     | 5 (1.5)                    | N/A          |
| Premature gonadal failure                      | 4 (1.2)                    | N/A          |
| Diabetes                                       | 4 (1.2)                    | N/A          |

SLE, Systemic lupus erythematosus; SLEDAI-2K, SLE disease activity index 2000; SLICC/ACR, Systemic Lupus International Collaborating Clinics/American College of Rheumatology; eGFR, estimated glomerular filtration rate; N/A, not applicable.

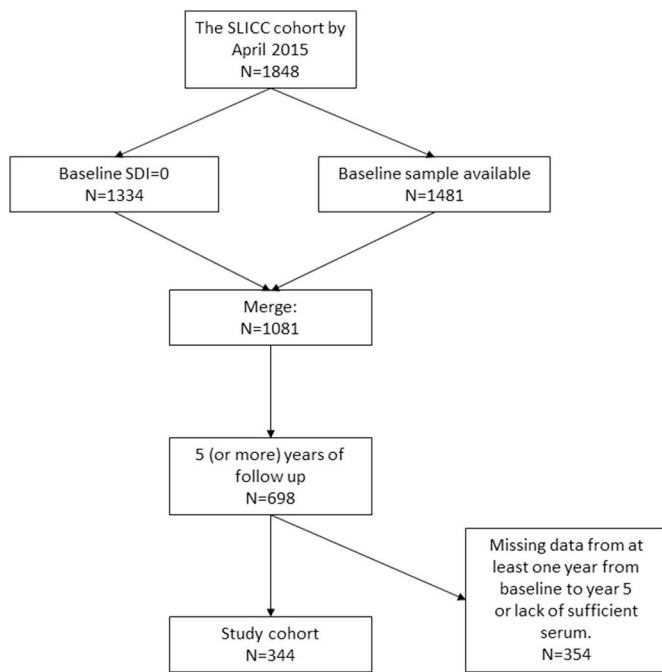


Fig. 1. Flow chart of the study population originating from the SLICC inception cohort.

Caucasians. The frequency of patients with any organ damage (SDI  $\geq 1$ ) 3 years after inclusion was 18.3%, whereas 28.5% of the patients had acquired damage at 5 years of follow-up. Detailed characteristics of the patients and controls are listed in Table 1. The ANA fine-specificities are presented in Supplementary Table 1.

### 3.2. suPAR concentrations and baseline variables

In baseline samples, the circulating levels of suPAR did not differ significantly between the patients (mean, 3.52 ng/mL; 95% CI 3.24–3.79 ng/mL) and the controls (mean, 3.57 ng/mL; 95% CI 3.39–3.75 ng/mL) (Fig. 2A). Likewise, there were no significant differences in suPAR levels regarding sex or race/ethnicity (Caucasians vs. non-Caucasians) among the patients or controls, and there was no difference when the patients were divided into Asians vs. non-Asians or divided into four groups (Caucasians, Asians, African ancestry, and Others). No significant differences in suPAR were found in relation to baseline treatment with antimalarials or immunosuppressants. Furthermore, no significant differences were found when comparing patients with low C3 and/or low C4 levels and patients with normal complement levels, and no significant differences were found based on ACR criteria (fulfilled at diagnosis), disease manifestations (SLEDAI-2K descriptors), autoantibody positivity (any autoantibody), anti-dsDNA positivity or other specific autoantibody positivity with a frequency of  $> 5\%$  (Supplementary Table 1). A significant correlation was found between age and suPAR level among the patients ( $p = 0.043$ ,  $r = 0.11$ ) and among the controls ( $p = 0.009$ ,  $r = 0.14$ ), and there was an inverse correlation between the eGFR and suPAR values ( $p < 0.001$ ,  $r = -0.20$ ) among the patients. A significant difference in suPAR levels was also found depending of the normality of eGFR ( $p = 0.001$ ), with higher levels of suPAR detected in patients with an eGFR  $< 90$  mL/min/1.73 m<sup>2</sup> (4.50 ng/mL; 95% CI 3.81–5.18 ng/mL), as compared with patients with normal eGFR (3.22 ng/mL; 95% CI 2.95–3.50 ng/mL). No statistically significant correlations were found between suPAR and cumulative SLEDAI-2K or any of the following baseline variables: SLEDAI-2K, anti-dsDNA levels, the number of autoantibody specificities, corticosteroid dose, ESR, CRP or disease duration (months from diagnosis to study inclusion) in the patients at baseline. Furthermore,

no significant differences in use of antimalarials, immunosuppressants, or daily corticosteroid dose at inclusion were observed between patients with high and low suPAR levels (Supplementary Table 2).

### 3.3. suPAR as a predictor of damage accrual

A significant difference in serum suPAR levels was found when patients without accrued damage were compared to patients with damage (SDI  $\geq 1$ ) at the 3-year follow-up ( $p = 0.019$ ), 4-year follow-up ( $p = 0.018$ ) and 5-year follow-up ( $p = 0.008$ ) (Fig. 2B). The mean suPAR of patients with organ damage at the 5-year follow-up was 4.09 ng/mL (95% CI 3.45–4.72 ng/mL) whereas patients without organ damage had a mean suPAR of 3.29 ng/mL (95% CI 3.01–3.57 ng/mL). An SDI score of  $\geq 2$  at 5 years has previously been shown to be linked to an increase in the relative risk for death among patients with SLE [40]. Consequently, the patients were also divided into three groups with respect to damage acquisition at the 5-year follow-up (Fig. 2C). The suPAR levels for the patients who had acquired major damage (SDI  $\geq 2$ ) over the 5-year period ( $N = 32$ ; mean, 4.83 ng/mL; 95% CI 3.25–6.42) were 46.8% higher than those for the patients without damage development ( $N = 246$ ; mean, 3.29 ng/mL; 95% CI 3.01–3.57 ng/mL) ( $p = 0.004$ ). Patients with SDI = 1 did not differ significantly from the other groups ( $N = 66$ ; mean, 3.73 ng/mL; 95% CI 3.16–4.29).

Logistic regression analysis (Table 2) revealed a significant impact of suPAR on SDI outcome at 5 years when the patients were divided into groups with no damage (SDI = 0) and with SDI  $\geq 1$  (OR = 1.13, 95% CI 1.02–1.25). A higher damage cut-off (SDI  $\geq 2$ ) also revealed a significant impact of suPAR on damage development (OR 1.14, 95% CI 1.03–1.26). The statistical significance for suPAR remained after adjustments for baseline factors that are associated with suPAR (age and

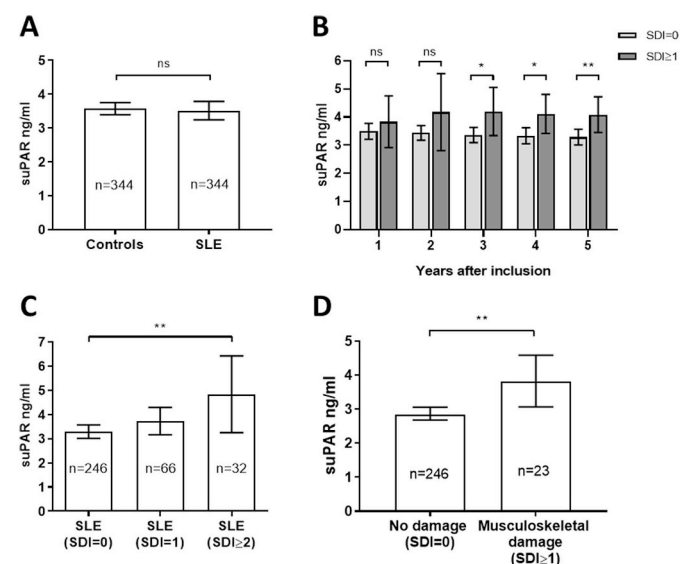


Fig. 2. Baseline suPAR levels in healthy controls and patients with different damage accrual. A) Healthy controls versus all patients. B) Baseline suPAR in relation to organ damage status at 1, 2, 3, 4 and 5 years post inclusion. C) Patients with different damage accrual at the 5-year follow-up. D) Patients with damage in the musculoskeletal domain compared to patients without organ damage development at the 5-year follow-up. Baseline suPAR was higher among patients with organ damage at 3, 4 and 5 years (Student's t-test). One-way ANOVA with Hochberg's post hoc test revealed higher baseline levels of suPAR in patients with organ damage accrual (SDI  $\geq 2$ ) at the 5-year follow-up compared with patients who did not suffer from organ damage (SDI = 0). suPAR levels in patients with musculoskeletal organ damage accrual at the 5-year follow-up were significantly higher than in patients without any damage development (Mann-Whitney U test). The bars and error bars indicate means (Panel A, B and C) or medians (Panel D) and 95% confidence intervals, respectively. \* $p < 0.05$ ; \*\* $p < 0.01$ ; ns = not significant.

**Table 2**Binary logistic regressions for the outcome of organ damage ( $SDI \geq 1$  and  $SDI \geq 2$ ) in patients with SLE at the 5-year follow-up.

| Model       | Cut-off          | AUC (95% CI)     | Variable (baseline) | OR (95% CI)      | p-value |
|-------------|------------------|------------------|---------------------|------------------|---------|
| Only suPAR  | ( $SDI \geq 1$ ) | 0.61 (0.54–0.67) | suPAR               | 1.13 (1.02–1.25) | 0.024   |
| Adjusted    | ( $SDI \geq 1$ ) | 0.61 (0.54–0.68) | suPAR               | 1.13 (1.01–1.26) | 0.027   |
|             |                  |                  | Age                 | 1.03 (1.01–1.05) | 0.013   |
|             |                  |                  | Abnormal eGFR       | N/A              | 0.166   |
| Optimized   | ( $SDI \geq 1$ ) | 0.65 (0.59–0.71) | suPAR               | 1.10 (1.00–1.21) | 0.047   |
|             |                  |                  | Age                 | 1.02 (1.00–1.04) | 0.023   |
|             |                  |                  | Male sex            | 2.45 (1.09–5.49) | 0.029   |
|             |                  |                  | SLEDAI-2K           | 1.06 (1.01–1.11) | 0.014   |
| Only suPAR  | ( $SDI \geq 2$ ) | 0.64 (0.54–0.74) | suPAR               | 1.14 (1.03–1.26) | 0.014   |
| Adjusted    | ( $SDI \geq 2$ ) | 0.68 (0.58–0.78) | suPAR               | 1.14 (1.03–1.26) | 0.014   |
|             |                  |                  | Age                 | 1.04 (1.01–1.07) | 0.014   |
|             |                  |                  | Abnormal eGFR       | N/A              | 0.208   |
| Optimized A | ( $SDI \geq 2$ ) | 0.74 (0.65–0.83) | suPAR               | 1.15 (1.04–1.27) | 0.006   |
|             |                  |                  | Age                 | 1.04 (1.02–1.07) | 0.003   |
|             |                  |                  | Non-Caucasian       | 2.50 (1.07–5.83) | 0.035   |
|             |                  |                  | Corticosteroid dose | 1.03 (1.00–1.05) | 0.019   |
| Optimized B | ( $SDI \geq 2$ ) | 0.77 (0.69–0.86) | suPAR               | 1.13 (1.02–1.25) | 0.017   |
|             |                  |                  | Age                 | 1.04 (1.02–1.07) | 0.001   |
|             |                  |                  | Non-Caucasian       | 2.50 (1.07–5.83) | 0.024   |
|             |                  |                  | SLEDAI-2K           | 1.03 (1.00–1.05) | < 0.001 |

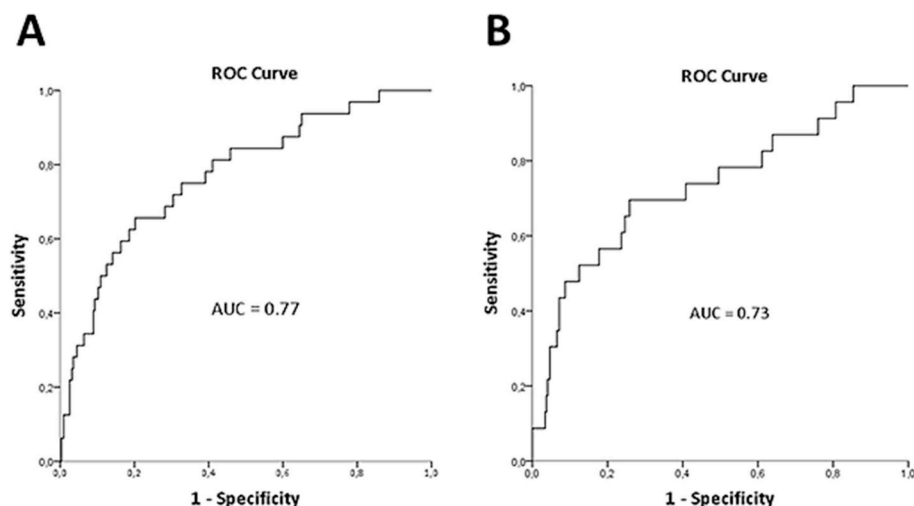
Abbreviations used: suPAR, soluble urokinase plasminogen activator receptor; eGFR, estimated glomerular filtration rate; SLEDAI-2K, systemic lupus erythematosus disease activity index 2000; N/A, not applicable.

eGFR) at both SDI cut-offs (adjusted models in Table 2).

Factors previously known to influence SDI [1,7,8], which were available in this study, were tested one by one as independent variables in a logistic regression with damage accrual ( $SDI \geq 1$  or  $SDI \geq 2$ ) at 5 years as the outcome variable. At the low SDI cut-off, age (OR = 1.02, 95% CI 1.01–1.04), male sex (OR = 2.57, 95% CI 1.19–5.54), baseline SLEDAI-2K (OR = 1.05, 95% CI 1.00–1.10) and cumulative SLEDAI-2K (OR = 1.02, 95% CI 1.00–1.04) had significant impacts on damage outcome. At the high SDI cut-off, baseline SLEDAI-2K (OR = 1.12, 95% CI 1.05–1.19), cumulative SLEDAI-2K (OR = 1.03, 95% CI 1.01–1.05), baseline corticosteroid dose (OR = 1.03, 95% CI 1.01–1.05), non-Caucasian ethnicity (OR = 2.53, 95% CI 1.19–5.35), and age (OR = 1.03, 95% CI 1.01–1.06) all had significant impacts on the damage outcome. Neither eGFR (as continuous or binary variable) nor CRP levels (N = 258) were associated with the damage outcome, regardless of the SDI cut-off. Variables that were significantly associated with the SDI outcome (when tested one by one) were then added as independent variables in a multiple logistic regression model with the respective SDI cut-offs (Table 2). Baseline SLEDAI-2K and corticosteroid dose were not added to the same model owing to their close relationship and to avoid overfitting the regression model. Cumulative SLEDAI-2K was not used in the regression models at all due to its high correlation with baseline

SLEDAI-2K ( $p < 0.001$ ,  $r = 0.641$ ). In addition, cumulative SLEDAI-2K is not a baseline variable and therefore not applicable as an early predictor of organ damage development. When the predicted probabilities from the regression models were used to create ROC curves, the highest AUC was found for the model with high SDI cut-off and with SLEDAI-2K, age, ethnicity and suPAR as independent variables (AUC = 0.77; Fig. 3A and Table 2). Autoantibody positivity (general or specific) was not associated with damage development in our study, except for associations between anti-ribosomal P protein positivity and  $SDI \geq 1$  and between anti-La/SSB positivity and  $SDI \geq 2$  (Supplementary Table 1).

Dissecting SDI into specific organ domains revealed significant differences in suPAR levels ( $p = 0.015$ ) between the patients with musculoskeletal damage (N = 23; median, 3.81 ng/mL; 97% CI 3.07–4.59) and patients without musculoskeletal damage (N = 321; median, 2.94 ng/mL; 96% CI 2.76–3.14), as well as when the group with musculoskeletal damage was compared with the group without any acquired damage at 5 years (N = 246; median 2.85 ng/mL; 95% CI 2.68–3.06;  $p = 0.007$ ) (Fig. 2D). The most common forms of musculoskeletal damage were muscle atrophy/weakness (N = 6), avascular necrosis (N = 6), and deforming/erosive arthritis (N = 5). A binary logistic regression analysis (Table 3) revealed a significant impact of suPAR on SDI outcome ( $SDI \geq 1$ ) in the musculoskeletal domain



**Fig. 3.** Receiver operating characteristic (ROC) curves showing the area under the curve (AUC) for predicted probabilities from the optimized logistic regression models. A) Optimized model for global damage accrual over 5 years ( $SDI \geq 2$ ) with baseline suPAR, SLEDAI-2K, age and ethnicity (Caucasian vs non-Caucasian) as independent variables. B) Optimized model for musculoskeletal damage accrual ( $SDI \geq 1$ ) over 5 years. The independent variables in this model were baseline suPAR, SLEDAI-2K, and age. Ethnicity (Caucasian versus non-Caucasian) was included in the model predicting global organ damage (A), but not in the model predicting musculoskeletal damage (B).

**Table 3**Binary logistic regressions for the outcome of organ damage in the musculoskeletal domain (SDI  $\geq 1$ ; yes/no) at the 5-year follow-up.

| Model      | AUC (95% CI)     | Variable (baseline) | OR (95% CI)      | p-value |
|------------|------------------|---------------------|------------------|---------|
| Only suPAR | 0.65 (0.54–0.76) | suPAR               | 1.13 (1.02–1.25) | 0.019   |
| Adjusted   | 0.65 (0.52–0.77) | suPAR               | 1.15 (1.04–1.28) | 0.022   |
|            |                  | Age                 | N/A              | 0.052   |
|            |                  | Abnormal eGFR       | N/A              | 0.557   |
| Optimized  | 0.73 (0.62–0.85) | suPAR               | 1.12 (1.01–1.65) | 0.032   |
|            |                  | Age                 | 1.04 (1.01–1.08) | 0.012   |
|            |                  | SLEDAI-2K           | 1.14 (1.06–1.23) | < 0.001 |

Abbreviations used: suPAR, soluble urokinase plasminogen activator receptor; eGFR, estimated glomerular filtration rate; SLEDAI-2K, systemic lupus erythematosus disease activity index 2000; N/A, not applicable.

(OR = 1.13; 95% CI: 1.02–1.25), also when adjusting for age and eGFR (OR = 1.15; 95% CI 1.04–1.28). Variables that were significantly associated with musculoskeletal damage when added one by one in binary regression analyses were subsequently added to a multiple regression analysis to create an optimized model for the prediction of damage in the musculoskeletal domain (Table 3). The predicted probabilities from this regression model were used to create a ROC curve (AUC = 0.73; Fig. 3B).

All the other groups of domain-specific organ damage, except for the neuropsychiatric domain, had higher median values of suPAR compared to patients without any damage (SDI = 0), although none of the comparisons met statistical significance.

#### 4. Discussion

suPAR has been proposed as a valuable biomarker that reflects severity in cases of malignancy, as well as inflammatory diseases including SLE [8,11,27,29,30]. For the first time, we report that suPAR has the potential to serve as a *predictor* of future global organ damage in patients with newly diagnosed SLE.

The suPAR levels at baseline were associated with global damage at the 5-year follow-up, particularly when using SDI  $\geq 2$  as the cut-off. No correlation was found with disease activity (SLEDAI-2K) at baseline, and no associations were found with the presence of autoantibodies included in the ‘immunological disorder criterion’ of the ACR classification (of which anti-dsDNA often parallel the disease activity), indicating that suPAR is disconnected from disease activity. This is in line with our previous observations of suPAR in SLE [8]. In other conditions, suPAR has been reported to: (1) predict cardiovascular morbidity and mortality (independent of traditional risk factors) [41]; (2) be associated with subclinical cardiovascular damage [29]; (3) predict a decline in eGFR [42]; and (4) be associated with decreased liver function [43]. Herein, the only separate SDI domain for which suPAR exerted a significant impact on damage outcome was the musculoskeletal domain, which includes muscle atrophy/weakness, deforming/erosive arthritis, osteoporosis, avascular necrosis, osteomyelitis, and tendon rupture [34]. Musculoskeletal damage was the second-most frequent type of damage in the study cohort, and since breakdown of damage into specific organ systems reduces the statistical power, an association between suPAR and damage accrual in other specific organ systems cannot be excluded. Studies on suPAR in patients with rheumatoid arthritis (RA) have shown higher suPAR levels compared with controls, as well as a correlation with the number of swollen joints [26,44]. In support of this, other groups have reported on the ability of synovial neutrophils from patients with RA to produce the chemotactically active form of suPAR (D2D3), thereby recruiting leukocytes to the joint [45]. Taken together, these findings suggest an important role for suPAR in joint inflammation and subsequent damage, which may provide mechanistic support for the association between suPAR and musculoskeletal damage found in the present study.

The cellular expression of uPAR in inflammatory diseases is poorly characterised. We have previously observed an association of reduced

suPAR levels and leukocytopenia in SLE [8], although leukocyte count data were unfortunately not available herein. Thus, the comparable levels of suPAR in SLE and matched controls might be attributable to the lack of adjustment for leukocyte counts. A previous large-scale study on suPAR has showed that the circulating levels increased slightly with age and were highest among women in a Caucasian population [46]. The influence of ancestry is less well-studied, although higher levels of suPAR have been observed among African compared to Caucasian males [47], whereas another study found no ethnicity-dependent differences in suPAR levels [42].

The potential biologic roles of suPAR in organ damage development have primarily been investigated in renal disease, where suPAR has been implicated in the onset and progression of focal segmental glomerulosclerosis [16]. The binding of suPAR to  $\beta 3$  integrins on the podocyte membrane has been shown to affect podocyte behaviour and, thereby, disrupt the glomerular barrier function [16].

Since the pathogenesis of SLE is characterised by impaired clearance of dying cells, it is of interest to highlight the involvement of uPAR in the disposal of dying cells. Both uPAR and suPAR have been shown to regulate the phagocytosis of apoptotic cells [15,48], and uPAR deficiency may lead to the accumulation of cell debris [49]. Interestingly, exclusively one-sided expression of uPAR on murine macrophages or neutrophils has been shown to increase the uptake of apoptotic neutrophils by the macrophages [48]. uPAR has also been proposed as a mediator of cardiac neonatal lupus based on its upregulation following the binding of anti-Ro60 antibodies to foetal cardiocytes [14,50]. Increased expression of uPAR has been shown to reduce directly the uptake of apoptotic myocytes by healthy myocytes. Ro60/SSA-dependent upregulation of uPA/uPAR can also facilitate plasmin-dependent activation of transforming growth factor (TGF)- $\beta$  to promote fibrosis, which is interesting in the context of organ damage [51]. In the present study, however, no association between anti-Ro60/SSA autoantibodies and suPAR was found.

SLE is indeed a heterogeneous disease and in the era of precision medicine clinicians will need better tools to facilitate the risk-stratification and enable a treat-to-target approach [52]. Based on the data presented here, suPAR analysis may contribute to the identification of patients in risk of developing severe disease, at least over the first 5 years. We suggest that raised suPAR levels, irrespective of SLE disease activity, should lead to increased vigilance and monitoring. This may not necessarily result in additional visits to the rheumatologist but could motivate an increased frequency of blood and urine sampling, and encourage a re-evaluation of the prescribed daily corticosteroid dose. In addition, it could motivate the analysis of circulating hydroxychloroquine levels to reassure patient's compliance in order to reach adequate blood levels and provide the best possible prerequisites to avoid future organ damage. The latter has repeatedly been shown to associate with both poor quality-of-life and survival [40,53,54].

The present study has several strengths. It has a prospective design and employs a large international inception cohort of patients with SLE with 5 years of follow-up data. This international inception cohort is unique in terms of the number of incident SLE cases, and the study

population is extremely well-characterised. To reduce biases linked to previous damage, which is associated with further damage [55], only those patients who had no damage at inclusion were included in the present study.

The limitations of the study include the differences in ethnicity between patients and controls. However, the suPAR levels did not differ significantly between the various ethnicities in our study. Although patients were recruited early in their disease course with an average time of 5 months from diagnosis to study inclusion, a considerable proportion had received antimalarials and/or immunosuppressants by the time of blood sampling. Nevertheless, the impact of pharmacotherapy, including the effects of corticosteroids, on suPAR levels appeared to be minor (Supplementary Table 2). While the relatively low number of damage events over 5 years probably reflects well-controlled patients, it also generates uncertainties regarding the prediction of damage accrual. A longer follow-up of these patients might clarify whether suPAR is associated with damage development in specific organ domains. One should also consider that the predictive value of suPAR potentially could vary over time in established SLE. Finally, we included only those patients who had completed follow-up over the 5 years. A minor survivor bias cannot be excluded, since the patients who died or were lost to follow-up did not contribute to the analysis.

## 5. Conclusion

In conclusion, suPAR is herein shown to be a predictor of global organ damage accrual over 5 years in cases of recent-onset SLE. Thus, this easy-to-measure soluble receptor has strong potential as a risk-stratifying biomarker that can identify those patients who will need careful monitoring, irrespective of disease activity.

## Declaration of competing interest

Dr. Petri reports grants and personal fees from AstraZeneca, GSK and Exagen, personal fees from Abbvie, Aleon Pharmaceuticals, Amgen, Astellas, Blackrock Pharmaceuticals, Bristol-Myers Squibb, Decision Resources, EMD Serono, Eli Lilly, Glenmark Pharmaceuticals, IQVIA, Janssen Pharmaceuticals, INOVA Diagnostics, Kezar Life Sciences, Medscape LLC, Momenta Pharmaceuticals, Novartis Pharmaceuticals, Principia Biopharma, Qiagen, and UCB Pharmaceuticals, outside the submitted work; Dr. van Vollenhoven reports grants from BMS, GSK, Lilly, Pfizer, Roche, UCB, personal fees from AbbVie, Biotest, BMS, Celgene, GSK, Janssen, Lilly, Pfizer, Servier, and UCB, outside the submitted work; Dr. Clarke reports consultant fees from AstraZeneca, Bristol Myers Squibb, and Exagen Diagnostics, outside the submitted work.

## Acknowledgments

We thank the EIRA study personnel for providing us with information and sera from healthy controls. Nicole Anderson and Anne MacKinnon are acknowledged for biobank handling and data management.

This study was funded by grants from the Swedish Rheumatism Association, the Region Östergötland (ALF Grants), the King Gustaf V's 80-year Anniversary Foundation, and the King Gustaf V and Queen Victoria's Freemasons Foundation. Dr. Bruce is a National Institute for Health Research (NIHR) Senior Investigator and is funded by Versus Arthritis, the NIHR Manchester Biomedical Research Centre, and the NIHR/Wellcome Trust Manchester Clinical Research Facility. Dr. Caroline Gordon is supported by Lupus UK, the Sandwell and West Birmingham Hospitals NHS Trust, and the National Institute for Health Research (NIHR)/Wellcome Trust Birmingham Clinical Research Facility. The views expressed are those of the author(s) and are not necessarily those of the NHS, the NIHR or the Department of Health. Rosalind Ramsey-Goldman's work was supported by the NIH (grants

8UL1TR000150, formerly UL-1RR-025741, K24-AR-02318 and P60AR064464, formerly P60-AR-48098). Dr. Sang-Cheol Bae's work was supported in part by funding (NRF-2017M3A9B4050335) from the National Research Foundation of Korea.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaut.2019.102340>.

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