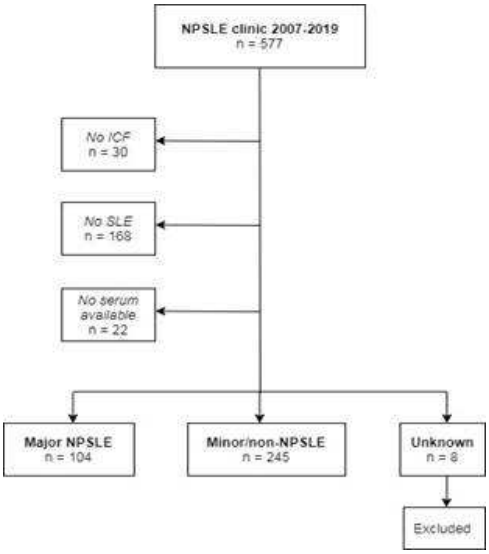
SUPPLEMENTARY FILES Part I: Additional cohort information

Supplementary Figure 1 Patient inclusion. The diagram illustrates the number of patients that were included and excluded from this study. The resulting number of patients include patients from whom SLE has been confirmed and required data was available. Namely, 104 patients with major NPSLE and 245 minor/non-NPSLE, making a total of 349 patients.



ICF = informed consent form; *NPSLE* = neuropsychiatric systemic lupus erythematosus

Supplementary Table 1 NPSLE syndromes (n = 176) according to 1999 ACR criteria of patients with major NPSLE (n = 104)

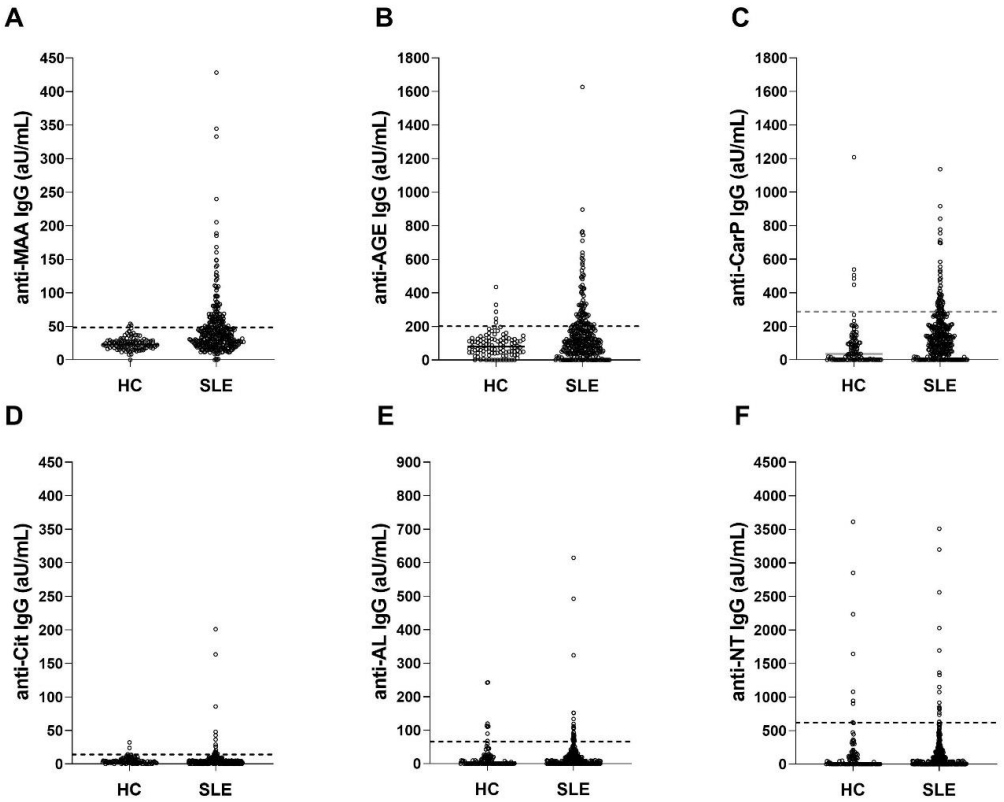
NPSLE syndrome (n, % of patients with major NPSLE)	
Aseptic meningitis	1 (1)
Cerebrovascular disease	45 (43)
Demyelinating syndrome	0 (0)
Headache	8 (8)
Movement disorder (chorea)	3 (1)
Myelopathy	9 (9)
Seizure disorders	9 (9
Acute confusional state	8 (8)
Anxiety disorder	1 (1)
Cognitive dysfunction	39 (38)
Mood disorder	13 (13)
Psychosis	7 (7)
AIDP[b]	0 (0)
Autonomic disorder	0 (0)
Mononeuropathy	0 (0)
Myasthenia gravis	0 (0)
Neuropathy, cranial	6 (2)
Plexopathy	0 (0)
Polyneuropathy	4 (4)
Other[c]	31 (30)

[a] Patients with neuropsychiatric symptoms attributed to SLE

[b] Acute inflammatory demyelinating polyneuropathy.

[c] Other NPSLE symptoms: cerebral vasculitis (n = 15), organic brain syndrome (n = 3), lethargia (n = 1) visual disturbance other than optic neuritis (n =2), apraxia (n =2), apathy (n = 2) walking disorder (n =2), motor disorder left arm (n =1), paresis left arm and dysarthria (n = 1), increased intracranial pressure (n =1), mononeuritis multiplex (n =1).

Supplementary Figure 2 Levels of different IgG antibodies against specific post-translational modifications (A) MAA, (B) AGE, (C) CarP, (D) Cit, (E) AL and (F) NT in healthy controls (HC) and patients with systemic lupus erythematosus (SLE). Reactivity was determined using ELISA and cut-off was calculated using mean + plus two times the SD of the healthy controls (dashed line), as described in the Material and Methods. Reactivity is depicted as arbitrary units per milliliter (aU/mL). AGE, advanced glycation end-product; AL, acetylated protein; CarP, carbamylated protein; Cit, citrullinated protein; MAA, malondialdehyde-acetaldehyde adduct; NT, nitrated protein.



Supplementary Table 2 Details regarding correlation coefficients (including 95% confidence intervals) provided in Figure 2

	Anti-MAA		**Anti-AGE**		**Anti-CarP**	
		95% CI*	corr	95% CI	corr	95% CI
Demographics						
Age	-0.09	-0.19; 0.02	-0.02	-0.13; 0.09	0.13	0.03; 0.23
Disease duration	-0.17	-0.27; -0.07	-0.10	-0.20; 0.01	-0.02	-0.12; 0.09
Disease activity and damage						
SLEDAI	0.12	0.01; 0.22	0.18	0.08; 0.28	0.09	-0.02; 0.19
SDI	-0.02	-0.12; 0.09	0.02	-0.09; 0.12	0.05	-0.15; 0.06
Complement factors						
C1q	-0.08	-0.19; 0.02	-0.09	-0.19; 0.02	0.05	-0.06; 0.15
C3	-0.20	-0.30; -0.09	-0.17	-0.28; -0.06	-0.04	-0.15; 0.07
C4	-0.24	-0.35; -0.13	-0.19	-0.30; -0.08	-0.07	-0.18; 0.04
Inflammation						
ESR	0.30	0.20; 0.39	0.19	0.08; 0.28	0.10	0.00; 0.20
CRP	0.19	0.09; 0.30	0.10	0.00; 0.20	0.07	-0.04; 0.17
Antibodies						
ANA	0.12	0.02; 0.22	0.12	-0.01; 0.22	0.04	**-0.06; 0.15**
Anti-dsDNA	0.25	0.15; 0.35	0.15	0.05; 0.25	0.05	-0.06; 0.15
Anti-SSA	0.02	-0.09; 0.12	0.04	-0.07; 0.15	0.09	-0.02; 0.19
Anti-SSB	0.05	-0.09; 0.12	0.04	-0.07; 0.14	0.04	-0.07; 0.14
Anti-SM	0.13	-0.02; 0.23	0.12	0.02; 0.23	0.06	-0.04; 0.17
Anti-RNP	0.08	-0.03; 0.18	0.05	-0.06; 0.15	0.00	-0.11; 0.10
Anti-cardiolipin	0.19	0.09; 0.29	0.07	-0.03; 0.18	-0.02	-0.12; 0.09
Anti-B2-GP1	0.02	-0.10; 0.14	0.02	-0.10; 0.14	0.01	-0.11; 0.12
LAC	0.09	-0.02; 0.19	0.03	-0.07; 0.14	-0.03	-0.13; 0.08

All confidence intervals were calculated using Fisher's transformation

*Correlation between anti-MAA IgG, anti-AGE IgG and anti-CarP IgG and clinical and laboratory markers in patients with systemic lupus erythematosus. Measured by Spearman correlation analyses (demographics – inflammation) and point biserial correlation analyses after transformation (antibodies).

AGE, advanced glycation end-product; ANA, anti-nuclear antibodies; anti-B2-GP1, anti-beta-2-glycoprotein; anti-dsDNA, anti-double stranded DNA; anti-RNP, anti-ribonucleoprotein; anti-SM, anti-Smith; anti-SSA/B, anti–Sjögren's-syndrome-related antigen A/B autoantibodies; CarP, carbamylated protein; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LAC = lupus anticoagulant; MAA, malondialdehyde-acetaldehyde adduct; SLEDAI, systemic lupus erythematosus disease activity index; SLICC, systemic lupus International Collaborating Clinics damage index.

Supplementary Table 3 The association between anti-PTMs (IgG) and major organ manifestations according to the 1997 ACR criteria for SLE[25]

	Renal disease		**Arthritis**		**Serositis**	
	Yes	No	Yes	No	Yes	No
	n = 94	*n = 255*	*n = 206*	*n = 143*	*n = 90*	*n = 259*
Anti-MAA						
aU/mL	31 [23-45]	37 [23-56]	38 [24-56]	31 [21-50]	38 [24-56]	32 [22-51]
Positive	18 (19)	83 (33)	63 (31)	38 (27)	27 (30)	74 (29)
Anti-AGE						
aU/mL	104 [47-190]	115 [52-102]	112 [46-209]	114 [55-192]	128 [59-215]	110 [47-187]
Positive	14 (15)	49 (19)	22 (15)	41 (20)	20 (22)	43 (17)
Anti-CarP						
aU/mL	133 [70-186]	125 [44-212]	123 [50-204]	130 [50-212]	138 [68-209]	122 [50-205]
Positive	11 (12)	38 (15)	18 (13)	31 (15)	16 (18)	33 (13)

Results are presented as n (%) or median [IQR]. Levels are AU/mL

AGE, advanced glycation end-product; CarP, carbamylated protein; MAA, malondialdehyde-acetaldehyde adduct.

Supplementary Table 4 The association between anti-PTMs (IgG) and specific NPSLE phenotypes

	Major NPSLE		
	Ischemic	Inflammatory	Combined phenotype
	n = 28	*n = 51*	*n = 25*
Anti-MAA			
aU/mL	29 [20 – 64]	44 [27 – 57]	39 [24 – 65]
Positive	10 (36)	21 (41)	10 (40)
Anti-AGE			
aU/mL	111 [37 – 84]	154 [70 – 253]	125 [44 – 260]
Positive	3 (11)	14 (27)	7 (28)
Anti-CarP			
aU/mL	90 [41 – 245]	137 [55 – 209]	182 [54 – 323]
Positive	6 (21)	7 (14)	8 (32)

Results are presented as n (%) or median [IQR].

AGE, advanced glycation end-product; CarP, carbamylated protein; NPSLE, neuropsychiatric systemic lupus erythematosus; MAA, malondialdehyde-acetaldehyde adduct.

Supplementary Table 5 The association between anti-PTMs (IgG) and brain volumes (n = 182)

	Grey matter	**White matter**	**Total**	**White matter hyperintensity**
	r_s	r_s	r_s	r_s
Anti-MAA	-0.13 (-0.27; 0.01)	-0.20 (-0.34; -0.06)	-0.18 (-0.31; -0.03)	-0.06 (-0.20; 0.09)
Anti-AGE	-0.13 (-0.27; 0.02)	-0.16 (-0.30; -0.02)	-0.16 (-0.29; -0.01)	0.06 (-0.09; 0.20)
Anti-CarP	-0.13 (-0.27; 0.01)	-0.14 (-0.28; 0.01)	-0.14 (-0.28; 0.01)	0.19 (0.05; 0.33)

Results are presented as Spearman R correlation coefficients (95% CI), as calculated by Spearman rank-order correlation.

AGE, advanced glycation end-product; CarP, carbamylated protein; MAA, malondialdehyde-acetaldehyde adduct; r_s, spearman rank coefficient.

SUPPLEMENTARY FILES Part II: Methods

Routine laboratory assessment

IgG anti-dsDNA antibodies were detected using the *Crithidia luciliae* indirect immune fluorescence technique (Immuno Concepts, Sacramento, CA, USA). IgG antibodies against SS-A/Ro-52, SS-B/La, Sm, RNP and IgG and IgM anticardiolipin (aCL) and anti-β2 glycoprotein 1 (anti-β2GP1) antibodies were determined using a Phadia® 250 EliA fluorescence enzyme immunoassay (FEIA) (Thermo Scientific, Freiburg, Germany). Anti-β2GP1 (IgG/IgM) and IgG antibodies against SS-A, SS-B, SM and RNP were considered positive if levels were >10 U/ml. aCL (IgG/IgM) was considered positive if levels were >30 GPL/U/ml. Lupus anticoagulant (LAC) was determined using STA-Rack and STA Evolution coagulation analyzers (Stago, Parsippany, NJ, USA). ANA analysis was performed with an immunofluorescence assay test on Hep-2 cells using a dilution of 1:40. ANA was defined as positive according to the normal limits of the LUMC laboratory (positive, 74% or weakly positive, 15%).

C1q, C3 and C4 were measured in serum using laser nephelometry. Based on the normal limits for our laboratory, C1q, C3 and C4 were defined as low or normal/high.

Erythrocyte sedimentation rate (ESR) was measured using the Westergren-method (StaRRsed Compact). C-reactive protein (CRP) was measured with turbidimetric assays using Roche COBAS 8000 Modular - c702. CRP <3 was under the detection limit until 2017, thereafter high sensitivity CRP was reported (hsCRP). The values of 2017 with CRP < 3 (no exact measurement) were imputed with the median of CRP < 3 after 2017 (exact measurement, median 0.8, range 0.3 – 2.5).

Generation of antigens

For all modifications backbone Fetal Calf Serum (FCS, Bodinco, Alkmaar, the Netherlands) was used. For malondialdehyde acetaldehyde adducts (MAA), first an MDA solution was prepared using 0.5M 1,1,3,3,-Tetramethoxypropane (108383, Sigma) and 0.3% hydrochloric acid (1003171000, EMSURE) and incubated for 12 minutes at 37C in a water bath. [31] Meanwhile 20mg/mL protein was prepared and diluted 1:5 with 20% MilliQ, 20% freshly prepared MDA solution, and 4% acetaldehyde (402788, Sigma), pH was adjusted to pH=4.8 and MilliQ was added till 5 times volume of protein was reached. The solution was then incubated for 2hr at 37C in a water bath. Advanced glycation end-products (AGE) were created by incubating 2mg/mL protein with 1:100 diluted 3.3M glycolaldehyde (23147-58-2, Sigma). [32] The solution was filtered using a 0.2um whatmann filter (10462200, GE Healthcare) and incubated for 10 days at 37C while shaking. For carbamylation (CarP) of proteins, first a 2M potassium cyanate (KOCN, 215074, Sigma-Aldrich) solution in PBS was prepared. [7] 4mg/mL protein

backbone was incubated to a 1:1 volume-to-volume ratio and incubated for 12hr at 37C. Citrullinated (Cit) proteins were created by incubating 1mg protein in a volume of 1mL containing 0.1M TRIS-HCl pH 7.6, 5mM DL-DTT (D0632, Sigma), 1mg/mL protein, 1U Peptidyl Arginine Deiminase 4 enzyme/100uL (PAD enzyme, 1584, Sigma) and CaCl2 10mM (21097, Sigma), volume was supplemented with MilliQ. [29] The mixture was incubated for 6hr at 53C. After incubation citrullinated protein was spun down and stored at -80C. Acetylation (AL) was performed using protein at a concentration of 1mg/mL in 0.1M Na2CO3. 50uL of acetic anhydride (100042, Merck) and 200uL of pyridine (109728, Merck) was added per 10mL of protein solution. [30] Proteins were incubated for 5hr at 30C. After incubation, the reaction was stopped using 200uL (per 10mL solution) of 1M TRIS. Samples were concentrated using Amicon Ultra – 15 centrifugal filter unit (10kDa, UFC901024, Sigma) and buffer was exchanged to PBS using Zeba Spin Desalting columns (89890, Thermofisher). For nitration (NT) 6 equal shots of peroxynitrite (14042-01-4, Cayman Chem) were directly used from stock solution to create an end concentration of 6mM. [28] After every shot the mix was directly vortexed for 30 seconds and left on ice for one minute before adding the next shot. After the last shot the samples were incubated for 1hr at 37C. Following incubation, all modifications (except for Cit, which was spun down) and their non-modified counterpart were extensively dialyzed against PBS using 10kDa cut-off SnakeSkin dialyzing tube (88243, Thermofisher). All modifications were stored at -20C until used. Protein concentrations were measured using Bradford solution (500-0006, Biorad) or Nanodrop. Modifications were verified using an ELISA based assay using anti-PTM specific antibodies: goat anti-MAA-HRP, ab20703, abcam; mouse anti-AGE 4B5, kindly provided by prof. H de Boer; rabbit anti-CBL, STA-078, biolabs; mouse anti-citrulline F95, MABN328, MilliPore; rabbit anti-acetyllysine, ADI-KAP-TF120-E, Enzo Lifesciences; mouse anti-nitrotyrosine, ab61392, abcam.

SUPPLEMENTARY FILES Part III: Results repeated for patients with positive anti-nuclear antibodies (ANA) at time of visit

Supplementary Table 6 Prevalence of antibodies against specific post-translational modifications in healthy control and patients with systemic lupus erythematosus with positive ANA

	SLE patients (n = 258)		Healthy controls (n = 108)		Median difference (95% CI)
	aU/mL	***n,% positive***		***n,% positive***	
Anti-MAA	39 [1 - 57]*	91 (35)	23 [18 - 29]	3 (3)	16 (10; 22)
Anti-AGE	125 [56 – 215]*	56 (22)	80 [41 – 122]	4 (4)	45 (11; 79)
Anti-CarP	126 [50 – 220]*	41 (16)	35 [0 – 11]	5 (5)	91 (55; 128)
Anti-Cit	3 [2 – 6]	14 (5)	3 [2 – 6]	3 (3)	0 (-1; 1)
Anti-AL	8 [0 – 26]	23 (9)	4 [0 – 19]	8 (7)	4 (-3; 10)
Anti-NT	46 [0 – 180]	10 (4)	10 [0 – 132]	8 (7)	36 (0; 72)

Results are presented as n (%) or median [IQR]. * p ≤ 0.0001

AGE, advanced glycation end-product; AL, acetylated protein; CarP, carbamylated protein; Cit, citrullinated protein; MAA, malondialdehyde-acetaldehyde adduct; NT, nitrated protein.

Supplementary Figure 3. Correlation between (A) anti-MAA IgG (B) anti-AGE IgG and (C) anti-CarP IgG and clinical and laboratory markers in ANA positive patients. Measured by Spearman correlation analyses (demographics – inflammation) and point biserial correlation analyses after transformation (antibodies). AGE, advanced glycation end-product; ANA, anti-nuclear antibodies; anti-B2-GP1, anti-beta-2-glycoprotein; anti-dsDNA, anti-double stranded DNA; anti-RNP, anti-ribonucleoprotein; anti-SM, anti-Smith; anti-SSA/B, anti–Sjögren's-syndrome-related antigen A/B autoantibodies; CarP, carbamylated protein; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LAC = lupus anticoagulant; MAA, malondialdehyde-acetaldehyde adduct; SLEDAI, systemic lupus erythematosus disease activity index; SLICC, systemic lupus International Collaborating Clinics damage index.