



HAL
open science

Clonal haematopoiesis of indeterminate potential and cardiovascular events in systemic lupus erythematosus (HEMATOPLUS study)

Clémence David, Nicolas Duployez, Philippine Eloy, Drifa Belhadi, Julie Cheznel, Véronique Le Guern, Cédric Laouénan, Laurène Fenwarth, Diane Rouzaud, Alexis Mathian, et al.

► To cite this version:

Clémence David, Nicolas Duployez, Philippine Eloy, Drifa Belhadi, Julie Cheznel, et al.. Clonal haematopoiesis of indeterminate potential and cardiovascular events in systemic lupus erythematosus (HEMATOPLUS study). *Rheumatology*, 2022, 61 (11), pp.4355-4363. 10.1093/rheumatology/keac108 . hal-03856560

HAL Id: hal-03856560

<https://u-picardie.hal.science/hal-03856560v1>

Submitted on 16 Jan 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Short Title: CHIP, SLE and cardiovascular events

Clonal hematopoiesis of indeterminate potential and cardiovascular events in systemic lupus erythematosus (HEMATOPLUS study)

Clémence David, MD¹, Nicolas Duployez, MD-PhD², Philippine Eloy, MD³, Drifa Belhadi, PhD³, Julie Chezel, MD¹, Véronique Le Guern, MD⁴, Cédric Laouéan, MD-PhD³, Laurène Fenwarth, MD-PhD², Diane Rouzaud, MD¹, Alexis Mathian, MD-PhD⁵, Sébastien de Almeida Chaves, MD⁶, Pierre Duhaut, MD-PhD⁷, Olivier Fain, MD-PhD⁸, Lionel Galicier, MD⁹, Pascale Ghillani-Dalbin, MD¹⁰, Jean Emmanuel Kahn, MD-PhD¹¹, Nathalie Morel, MD⁴, Laurent Perard, MD¹², Micheline Pha, MD⁵, Françoise Sarrot-Reynauld, MD¹³, Olivier Aumaitre, MD-PhD¹⁴, François Chasset, MD-PhD¹⁵, Nicolas Limal, MD¹⁶, Helene Desmurs-Clavel, MD¹⁷, Felix Ackermann, MD-PhD¹⁸, Zahir Amoura, MD⁵, Thomas Papo, MD¹, Claude Preudhomme, MD-PhD², Nathalie Costedoat-Chalumeau, MD-PhD⁴, Karim Sacre, MD-PhD¹

1-Département de Médecine Interne, Hôpital Bichat, Assistance Publique Hôpitaux de Paris (APHP), Institut national de la santé et de la recherche médicale (INSERM) U1149, Université de Paris, Paris, France

2- University of Lille, CNRS, Inserm, CHU Lille, Institut de Recherche contre le Cancer de Lille, UMR9020-UMR-S 1277-Canther-Cancer Heterogeneity, Plasticity and Resistance to Therapies, 59000 Lille, France

3-Département d'Epidémiologie et de Recherche Clinique, Hôpital Bichat, APHP, Université de Paris, Paris, France

4-APHP, Hôpital Cochin, Département de Médecine Interne, Centre de Référence Maladies Auto-immunes et Systémiques Rares, Paris, France; Université de Paris, CRESS, INSERM, INRA, Paris, France

5-Sorbonne Université, Assistance Publique–Hôpitaux de Paris, Groupement Hospitalier Pitié–Salpêtrière, French National Referral Center for Systemic Lupus Erythematosus,

Antiphospholipid Antibody Syndrome and Other Autoimmune Disorders, Service de Médecine Interne 2, Paris, France

6-Département de Médecine Interne, Hôpital Purpan, Centre Hospitalo-Universitaire (CHU) de Toulouse, Toulouse, France

7-Département de Médecine Interne, Hôpital Amiens Nord, CHU d'Amiens, Amiens, France

8-Département de Médecine Interne, Hôpital Saint Antoine, APHP, Université Pierre et Marie Curie, Paris, France

9-Département d'Immunologie Clinique, Hôpital Saint Louis, APHP, Université de Paris, Paris, France ; Département de Médecine Interne, Hôpital Saint Joseph, Marseille, France

10- Département de Immunologie, Hôpital Pitié-Salpêtrière, APHP, Université Pierre et Marie Curie, Paris, France

11-Département de Médecine Interne, Hôpital Ambroise Paré, APHP, Université de Versailles-Saint-Quentin en Yvelines, France

12-Département de Médecine Interne, Hôpital St Joseph St Luc, Lyon, France

13-Département de médecine interne, Hôpital Michallon, CHU de Grenoble Alpes, Grenoble, France

14- Département de médecine interne, Hôpital Gabriel-Montpied, CHU de Clermont-Ferrand, France

15- Département de médecine interne, Hôpital Tenon, APHP Université Pierre et Marie Curie, Paris, France

16- Département de médecine interne, Hôpital Henri Mondor, APHP Université Paris-Est Créteil, Paris, France

17- Département de médecine interne, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

18- Département de médecine interne, Hôpital Foch, Suresnes, France

Corresponding author : Pr Karim Sacre, Département de Médecine Interne, Hôpital Bichat, 46 Rue Henri Huchard, 75018 Paris, France. E-mail: karim.sacre@aphp.fr.

Study Funding: The PLUS study was funded by a grant from the French PHRC 2005 (number 05-125) Ministère de la Santé. The HEMATOPLUS study was funded by a grant from the French CRC 2018 (number 18-058) Assistance Publique – Hôpitaux de Paris. JC was supported by a research grant from the Fondation pour la Recherche Médicale (FRM DEA20170638077).

Conflict of interest: the authors declare that they have no conflict of interest.

List of collaborators (PLUS study group) to be added in pubmed

Leonardo Astudillo, Cristina Belizna, Nadia Belmatoug, Olivier Benveniste, Audrey Benyamine, Holly Bezanahary, Patrick Blanco, Benoît Brihay, Patrice Cacoub, Emmanuel Chatelus, Judith Cohen-Bittan Richard Damade, Eric Daugas, Christian de-Gennes, Jean-François Delfraissy, Céline Delluc, Aurélien Delluc, Alain Dupuy, Isabelle Durieu, Hang-Korn EA, Dominique Farge, Camille Frances, Christian Funck-Brentano, Frédérique Gandjbakhch, Justine Gellen-Dautremer, Bertrand Godeau, Cécile Goujard, Catherine Grandpeix, Claire Grange, Gaëlle Guettrot-Imbert, Loïc Guillevin, Eric Hachulla, Jean-Robert Harle, Julien Haroche, Pierre Hausfater, Jean Sebastien Hulot, Moez Jallouli, Jean Jouquan, Gilles Kaplanski, Homa Keshtmand, Mehdi Khellaf, Olivier Lambotte, David Launay, Du Le Thi Huong, Gaelle Leroux, Hervé Levesque, Olivier Lidove, Frederic Liote, Eric Liozon, Kim Ly, Matthieu Mahevas, Kubéraka Mariampillai, Xavier Mariette, Karin Mazodier, Marc Michel, Luc Mouthon, Rokiya Ngack, Jacques Ninet, Eric Oksenhendler, Jean-Luc Pellegrin, Olivier Peyr, Anne-Marie Piette, Jean-Charles Piette, Vincent Poindron, Fabienne Roux, David

Saadoun, Sabrinel Sahali, Laurent Sailer, Bernadette Saint-Marcoux, Yoland Schoindre, Jérémie Sellam, Damien Sene, Jacques Serratrice, Pascal Seve, Jean Sibia, Claude Simon, Amar Smail, Christelle Sordet, Jérôme Stirnemann, Benjamin Terrier, Salim Trad, Jean-François Viillard, Pierre-Jean Weiller, Noël Zahr.

ABSTRACT

Objective The detection of somatic mutations in genes of myeloid cells in asymptomatic patients - defining clonal hematopoiesis of indeterminate potential (CHIP) - predisposes to cardiovascular events (CVE) in the general population. We aimed to determine whether CHIP was associated with CVE in SLE patients.

Methods The study is an ancillary study of the randomized, double-blind, placebo-controlled, multicenter trial PLUS study conducted from June 2007 through August 2010 at 37 centers in France involving 573 SLE patients. The search for somatic mutations by high-throughput sequencing of 53 genes involved in clonal hematopoiesis was performed on genomic DNA collected at PLUS inclusion. The CHIP prevalence was assessed in SLE and in a retrospective cohort of 479 patients free of hematological malignancy. The primary outcome was the incident CVE in SLE.

Results Screening for CHIP was performed in 438 SLE patients (38 [29-47] years, 91.8% female). Overall, 63 somatic mutations were identified in 47 patients defining a CHIP prevalence of 10.7% in SLE. Most SLE patients (78.7%) carried a single mutation. Most variants (62.5%) were located in the *DNMT3A* gene. CHIP was associated with age, age at SLE diagnosis and a lower frequency of antiphospholipid antibodies. CHIP occurred more than 20-years earlier ($p < 0.00001$) in SLE than in controls. The detection of CHIP at inclusion was not associated with the occurrence of CVE during follow up (HR= 0.42 (0.06 – 3.21), $p=0.406$).

Conclusion The prevalence of CHIP is high in SLE with respect to age but was not associated with incident CVE.

KEYWORDS: clonal hematopoiesis of indeterminate potential; lupus; cardiovascular events

CLINICAL TRIAL REGISTRATION NUMBER NCT05146414

INTRODUCTION

Although mortality in systemic lupus erythematosus (SLE) has decreased significantly in the last decades,¹ the burden of cardiovascular disease remains a significant challenge.²⁻⁴ SLE patients display an accelerated atherosclerosis that strongly contributes to mortality,⁵ poorly explained by the traditional cardiovascular risk factors.⁶ Clonal hematopoiesis defines the clonal expansion of hematopoietic cells probably driven by a selective advantage given by leukemia-associated somatic mutations.⁷ Clonal hematopoiesis of indeterminate potential (CHIP) is found in asymptomatic adult patients with normal blood counts.⁸ CHIP correlates with age and predisposes to haematological malignancy,⁹ but is also causally associated with cardiovascular events (CVE) in the general population.^{10,11} In auto-immune diseases, such as systemic sclerosis¹² and rheumatoid arthritis,¹³ CHIP has been evaluated in a limited number of patients and was not associated with a particular phenotype. In SLE, the prevalence of CHIP is not known. The main objective of our study was to determine whether CHIP was associated with CVE in SLE patients.

PATIENTS AND METHODS

SLE Patients

The HEMATOPLUS study is an ancillary study of the previously published PLUS study.¹⁴ The PLUS Study was a randomized, double-blind, placebo-controlled, multicenter trial conducted from June 2007 through August 2010 at 37 centers in France involving 573 SLE patients. Details on the PLUS study methods including data collection and definitions of comorbid conditions have been previously published.¹⁴ The diagnosis of antiphospholipid syndrome (APS) was defined according to international criteria.¹⁵ Lupus nephritis diagnosis was based on International Society of Nephrology/Renal Pathology Society classification.¹⁶

All PLUS participants with available DNA were included. Among them, 14 further patients were excluded because of technical issues in Next Generation Sequencing (NGS), leaving 438 SLE patients analyzed (Figure S1).

Control group

For the control group, a retrospective cohort of 479 adult patients free of hematological malignancy was considered. These patients were part of 1833 patients who had been sequenced for myeloid malignancy-associated genes between January 2019 and January 2020 at the Laboratory of Hematology of CHU Lille. Physicians who performed the analyses prospectively registered final diagnoses during this period. Patients younger than 18 years old or with a diagnosis of acute myeloid leukemia, myelodysplastic/myeloproliferative neoplasms, myeloproliferative neoplasms, and myelodysplastic syndromes were excluded. The 479 remaining patients who did not meet sufficient criteria for a diagnosis of hematological malignancy were considered to have ICUS (idiopathic cytopenia of undetermined significance), CCUS (clonal cytopenia of undetermined significance), or CHIP (clonal hematopoiesis of indeterminate potential)¹⁷ (See Supplementary Materials for details).

Molecular analyses

Genomic DNA extracted from whole blood was studied by high-throughput sequencing of 53 genes associated with myeloid malignancies and/or clonal hematopoiesis. Libraries were prepared using the SureSelect XT system (Agilent Technologies) according to the manufacturer's instructions and run on NextSeq500 (Illumina). Raw data were analyzed with both Mutect2 (BroadInstitute) and SeqNext (JSI Medical System) and visualized with the homemade NGS report software v1 (Laboratory of Hematology, CHU Lille). A high depth of coverage (>1500 X) was obtained for all clonal hematopoiesis-associated genes and mutations were detected until a filtered variant allele frequency (VAF) of 1% was obtained, with at least 20 reads carrying the variant. Variant interpretation was performed considering minor allele frequencies (MAF) in the public GnomAD database of polymorphisms (variants with MAF > 0.02 in overall population/global ancestry or sub-continental ancestry were excluded) and VAF compatibility with a somatic state (retained variants displayed VAFs ranging from 1% to 34%). Additional in silico predictions were performed when possible. Frameshift and nonsense variants were always considered as relevant mutations. Single nucleotide variant effects on protein function were predicted using SIFT¹⁸ and PolyPhen-2.¹⁹ The effects of splicing variants were predicted with Human Splicing Finder version 3.1.

Primary Outcome

The primary outcome was the occurrence of cardiovascular events (CVE) over follow-up. Incident CVE were ascertained, blinded to NGS results, by physician interview using a standardized questionnaire and through examination of medical records. SLE patients with inadequate follow-up period (< 20 months) and past history of CVE at PLUS inclusion were excluded from the analysis (Figure S1). CVE included coronary heart disease (i.e. hospitalization for unstable angina, coronary revascularization, or, myocardial infarction),

stroke, revascularization procedure for other atherosclerotic cardiovascular diseases and sudden cardiac death. All CVE that occurred through March 2019 were considered for analysis. For patients who had more than one incident CVE, the first event was considered for analysis.

Ethical statement

The PLUS Study was approved by the Comité de Protection des Personnes, St Louis Hospital, Paris. The HEMATOPLUS study was approved by the Comité de Protection des Personnes SUD-EST II, Lyon. All participants gave written informed consent to participate at the time of study enrollment. The trial is registered with clinicaltrials.gov (NCT05146414).

Statistical analysis

Descriptive statistics were conducted; continuous variables were expressed as median [interquartile range] and categorical variables were expressed as numbers and percentages. For comparisons between groups, we used chi-square and Student's t tests, as appropriate. Univariate Cox models were used to evaluate the association between clinical, biological and therapeutic variables and the risk of CHIP at inclusion. The risk of CVE according to the CHIP status was analysed using the Kaplan–Meier method and groups were compared using a log-rank test. The statistical cluster analysis was performed following three steps: i) multiple correspondence analysis (MCA) to convert information into coordinates; ii) ascending hierarchical clustering (AHC) using the individual coordinates obtained with the previous MCA step; and iii) comparison of differences between clusters using chi-square tests. All tests were two-sided, and P values < 0.05 were considered to indicate a significant association. All analyses and figures were performed using R software version 3.6.0. Kaplan-Meier curves and the two-dimensional representation of multiple correspondence analysis were made with R software version 3.6.0, packages 'survminer' and 'factoextra'.

Role of the funding source

The funding source had no role in the study design; the collection, analysis, and interpretation of data, in the writing of the report; and in the decision to submit the paper for publication

RESULTS

Characteristics of SLE patients at PLUS inclusion

Among the 573 patients included in the PLUS study, 135 patients were excluded because of unavailable genomic DNA (n=121) or technical issues in NGS (n=14) leaving 438 SLE patients with NGS analysis (Figure S1). At inclusion, 402 (91.8%) patients were female; the median age was 38 [IQR: 29-47] years. Fifty-five (12.6%) were current smokers, 13 (3%) had high blood pressure, 45 (10.3%) had a BMI > 30 kg/m², 23 (5.3%) had dyslipidemia and eight (1.8%) had diabetes. In keeping with the exclusion criteria of the PLUS study, no patients had a chronic kidney disease (CKD) - as defined by an eGFR < 60 mL/min/1.73 m². None had hematological malignancy. The median duration of SLE disease at baseline was of eight [3-25-13] years and only 48 (11%) had a SLEDAI superior to four. Eighty-seven (19.9%) patients had a past-history of lupus nephritis. Phospholipid antibodies were found in 201 (45.9%) patients including 50 (11.4%) with an antiphospholipid syndrome. Hydroxychloroquine, steroids, immunosuppressive drugs, antiplatelet, biologics and, statin treatments had been prescribed in 433 (98.9%), 295 (67.4%), 169 (38.6%), 105 (24%), 28 (6.4%) and, 19 (4.3%) patients, respectively (Table 1).

Clonal hematopoiesis of indeterminate potential (CHIP) in SLE

Overall, 63 mutations in 53 genes associated with clonal hematopoiesis were identified in 47 patients without any evidence for hematological malignancy thus defining a CHIP prevalence of 10.7% (47 of 438 patients) in SLE patients. The prevalence of CHIP was also determined in

479 (66 [53-75] years, 50.7% females) controls (CTL) (Supplementary Materials). The frequency of CHIP was higher in controls than in SLE (21.1% vs 10.7%, $p < 0.00001$) consistent with the older age of CTL at time study (66 [53-75] years vs 38 [29-47] years, $p < 0.00001$). However, 85.1% ($n=40$ of 47 patients) of SLE patients with CHIP were younger than 60 while 90.6% ($n=96$ of 106 patients) of CTL with CHIP were older. Overall, SLE patients with CHIP were more than 20-years younger than CTL with CHIP (52 [41-57] years vs 74 [67-80], $p < 0.00001$) (Figure 1).

Thirty-seven (78.7%) of the 47 SLE patients with CHIP carried a single mutation. Five patients carried two distinct mutations, four carried three mutations and, one carried four mutations. Most mutations were located in the *DNMT3A* gene that accounted for 61.9% (39 of 63) of the mutations identified in 70.2% (33 of 47) of SLE patients with CHIP. Other mutations were in *TET2* (seven mutations identified in six patients), *GNAS* (three mutations in two patients), *ASLX1*, *TP53*, *SH2B3* (two mutations in two patients) and, *SETBP1*, *CBL*, *JAK2*, *PPM1D*, *ETV6*, *KDM6A*, *NFE2*, *SMC3* genes (one mutation in eight patients) (Figure 2 and Supplemental Table 1).

Determinants of CHIP in SLE

Univariate analysis showed that CHIP in SLE patients was associated with age at inclusion, age at SLE diagnosis, a European ethnic background, and a lower frequency of arthritis and, antiphospholipid antibodies (Table S2). Of note, no association was found with the use of immunosuppressive drugs at any time. Cluster analysis identified four clusters according to demographic characteristics, SLE features and CHIP (Table 2 and Figure S2). Cluster 1 was the largest one ($n=156$) and encompassed 74.5% ($n=35$ of 47) of SLE patients with CHIP. Patients in Cluster 1 had no history of lupus nephritis and no APS. Almost 50% ($n=126$ of 264) of patients with European descent aggregated in Cluster 1. Clusters 2 ($n=81$) and 3 ($n=122$)

encompassed only 2.1% (n= 1 of 47) and 6.4% (n=3 of 47) of patients with CHIP, respectively. More than 60% (n=31 of 50) of SLE patients with aPL aggregated in Cluster 2 while around 70% (n=47 of 69) of patients with Afro-American background aggregated in Cluster 3. Eventually, Cluster 4 (n=79) included almost 75% (66 of 89) of patients with lupus nephritis and 17% (n=8 of 47) of patients with CHIP.

CHIP and cardiovascular event in SLE patients

Among the 438 patients in whom clonal hematopoiesis was assessed, 333 could be analyzed for the primary outcome (Figure S1) with a median follow-up of nine [8-9] years. According to the Framingham score, the estimate absolute risk for cardiovascular events within the next ten years for the overall cohort was 0.1%.⁶ However, 19 (5.7%) patients experienced at least one cardiovascular event that occurred at a median of 4.8 [2.6-7.4] years after inclusion. Cardiovascular events included coronary heart disease (n=11) that required percutaneous coronary revascularization in all but one cases and nonfatal ischemic stroke (n=8). Four patients had more than one CVE. Kaplan-Meier analysis showed that the detection of CHIP at inclusion was not associated with the occurrence of CVE during follow up (HR= 0.42 [0.06 – 3.21], p=0.406) (Figure 3).

DISCUSSION

Our study shows that the prevalence of CHIP is high in SLE with respect to age. Indeed, clonal haematopoiesis was evidenced in more than 10% of SLE patients, despite a median age of 38. Although clearly associated with age, CHIP in SLE occurred before the age of 60 in most cases and more than 20 years earlier than in controls. It should be noticed that individuals in the control retrospective cohort should not be considered as healthy people since some of them had unexplained cytopenia (ICUS or CCUS). However, this would likely increase the prevalence

of clonal haematopoiesis in this population and therefore would not affect our conclusion. Comparison of CHIP prevalence between different series may be hazardous because of the heterogeneous sensitivity of the assays used to detect mutations.²⁰ Using an assay allowing to detect mutations with variant allele frequencies (VAF) as low as 0.001, clonal haematopoiesis was observed in 2.5% of the 20- to 29-year-old group, in 3.2% of the 30- to 39-year-old group, in 8.2% of the 40- to 49-year-old group and, in 13.2% of the 50- to 59-year-old group in a cohort of 2000 healthy controls.²¹ Noteworthy, with a VAF of 0.01 in our SLE cohort, clonal haematopoiesis was observed in 1.8% of the 20- to 29-year-old, in 6.8% of the 30- to 39-year-old, in 11.1% of the 40- to 49-year-old and, in 26.3% of the 50- to 59-year-old subgroups (Figure S3). In other systemic and autoimmune diseases such as systemic sclerosis,¹² rheumatoid arthritis¹³ or ANCA-associated vasculitis,²² prevalence of CHIP ranges from 14% to 30.4% of patients with a median age above 60 in all studies.

Noteworthy, CHIP in SLE was not associated with exposure to immunosuppressive drugs. The unexpectedly high CHIP prevalence observed in SLE supports the hypothesis of accelerated aging in SLE along with the facts that CVE occurs ten years earlier than in the general population,²³ the ability to respond properly to vaccines is reduced^{24,25} and, immunosenescent terminally differentiated T-cells are highly prevalent in SLE.^{26,27} The distribution of gene variants was comparable in SLE and in the general population⁹ suggesting that the disease itself did not confer a specific advantage for any somatic mutations in hematopoietic stem cells.

We found no association between CHIP and incident CVE in SLE. Consistent with a very low risk for CVE according to traditional cardiovascular risk factors and normal kidney function, the observed number of CVE was small. Moreover, only five patients had clonal variant frequencies greater than 10%, a threshold for which the association with CVE appears stronger

in the general population.²⁸ Eventually, while somatic *TET2* mutations in blood cells may play a causal role in atherosclerosis,^{10,11} most mutations were located in the *DNMT3A* gene in SLE. Interestingly, our cluster analysis identifies four SLE groups with distinct clinical phenotypes: 1 group of SLE patients without lupus nephritis that gathered the large majority of patients with CHIP; 2 groups of SLE patients displaying almost no CHIP, one group including the majority of APS patients and the other group the majority of Afro-American patients; and 1 last group of SLE patients that aggregated almost all patients with nephritis. Overall, CHIP in SLE may concern patients with a less severe disease consistent with the fact that the prevalence of lupus nephritis is lower in older patients.²⁹

Our study has several strengths. First it is the first assessing and describing a high prevalence of CHIP in SLE. Second, it is an ancillary longitudinal study of a large prospective and multicenter trial including more than 400 SLE with an extended follow up. Third, the unbiased samples strategy likely allows an accurate estimation of the prevalence of CHIP among SLE patients. Fourth, our primary endpoint did not focus on subclinical atherosclerosis but on incident CVE.

Our study also has several limitations. First, clonal hematopoiesis and primary outcome were not available for all participants. Second, primary outcome was not adjudicated. Third, the observed number of CVE was small, limiting statistical power. Last, patients with SLE and controls were not equally distributed for each age group.

In conclusion, although the prevalence of CHIP is high in SLE considering age, SLE patients with CHIP did not appear at higher risk for CVE.

KEY MESSAGES:

- Prevalence of clonal hematopoiesis of indeterminate potential (CHIP) is unexpectedly high in SLE with respect to age
- CHIP in SLE may concern patients with a less severe lupus disease.
- SLE patients with CHIP did not appear at higher risk for cardiovascular events.

ACKNOWLEDGEMENT

We are thankful to Mrs Ada Clarke for help with study organization and to Mrs Claire Fernandez for technical assistance

DATA SHARING

Data including individual participant data (clinical and CHIP sequencing) and a data dictionary defining each field in the set are available from the corresponding author upon reasonable request.

STUDY FUNDING:

The PLUS study was funded by a grant from the French PHRC 2005 (number 05-125) Ministère de la Santé. The HEMATOPLUS study was funded by a grant from the French CRC 2018 (number 18-058) Assistance Publique – Hôpitaux de Paris. JC was supported by a research grant from the Fondation pour la Recherche Medicale (FRM DEA20170638077).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

CD: formal analysis, investigation, methodology, writing – original draft, and writing– review & editing; ND: formal analysis, investigation, methodology, resources, and writing– review & editing; PE: formal analysis, methodology, project administration, supervision, validation, and writing– review & editing; DB: formal analysis, investigation, methodology, and writing– review & editing; JC: investigation, and writing– review & editing; VLG: methodology, project administration, resources, and writing– review & editing; CL: methodology, project administration, supervision, and writing– review & editing; LF: formal analysis, and writing– review & editing; DR: investigation, and writing– review & editing; AM: resources, and writing– review & editing; SAC: resources, and writing– review & editing; PD: resources, and writing– review & editing; OF: resources, and writing– review & editing; LG: resources, and writing– review & editing; PGD: resources, and writing– review & editing; JEK: resources, and writing– review & editing; NM: resources, and writing– review & editing ; LP: resources, and writing– review & editing; MP: resources, and writing– review & editing; FSR: resources, and writing– review & editing; OA: resources, and writing– review & editing; FC: resources, and writing– review & editing; NL: resources, and writing– review & editing; HDC: resources, and writing– review & editing; FA: resources, and writing– review & editing; ZA: resources, and writing– review & editing; TP: resources, and writing– review & editing; CP: investigation, methodology, resources, and writing– review & editing; NCC: data curation, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, and writing– review & editing; KS: conceptualisation, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, writing – original draft, and writing– review & editing.

CD, ND, PE, DB, and KS have directly accessed and verified the underlying data reported in the manuscript.

REFERENCES

1. Yen EY, Shaheen M, Woo JMP, Mercer N, Li N, McCurdy DK, et al. 46-Year Trends in Systemic Lupus Erythematosus Mortality in the United States, 1968 to 2013: A Nationwide Population-Based Study. *Ann Intern Med.* 2017 Dec 5;**167**:777–85.
2. Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine (Baltimore).* 2003 Sep;**82**:299–308.
3. Bartels CM, Buhr KA, Goldberg JW, Bell CL, Visekruna M, Nekkanti S, et al. Mortality and cardiovascular burden of systemic lupus erythematosus in a US population-based cohort. *J Rheumatol.* 2014 Apr;**41**:680–687.
4. Aviña-Zubieta JA, To F, Vostretsova K, De Vera M, Sayre EC, Esdaile JM. Risk of Myocardial Infarction and Stroke in Newly Diagnosed Systemic Lupus Erythematosus: A General Population-Based Study. *Arthritis Care Res (Hoboken).* 2017 Jun;**69**:849–856.
5. Thomas G, Mancini J, Jourde-Chiche N, Sarlon G, Amoura Z, Harlé J-R, et al. Mortality Associated With Systemic Lupus Erythematosus in France Assessed by Multiple-Cause-of-Death Analysis: Mortality and Systemic Lupus Erythematosus. *Arthritis & Rheumatology.* 2014 Sep;**66**:2503–2511.
6. Chezeli J, Costedoat-Chalumeau N, Laouénan C, Rouzard D, Chenevier-Gobeaux C, Le Guern V, et al. Highly sensitive serum cardiac troponin T and cardiovascular events in patients with systemic lupus erythematosus (TROPOPLUS study). *Rheumatology (Oxford).* 2020 Sep 9;
7. Challen G, Goodell MA. Clonal Hematopoiesis: Mechanisms Driving Dominance of Stem Cell Clones. *Blood.* 2020 Aug 3;

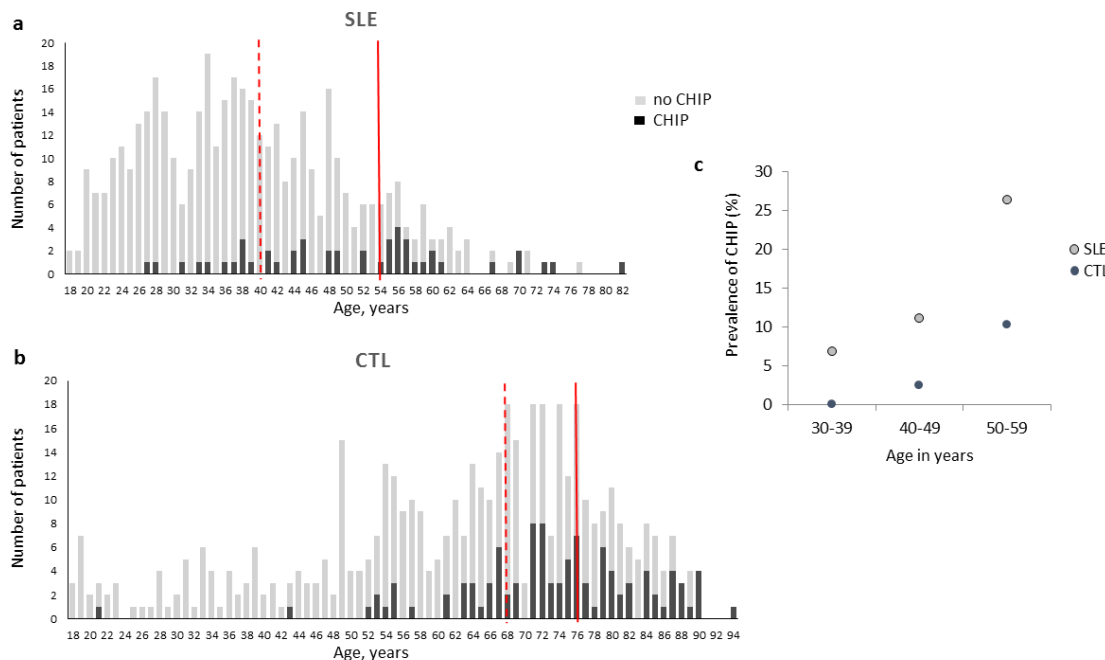
8. Shlush LI. Age-related clonal hematopoiesis. *Blood*. 2018 01;**131**:496–504.
9. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014 Dec 25;**371**:2488–98.
10. Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, et al. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N Engl J Med*. 2017 13;**377**:111–21.
11. Fuster JJ, MacLauchlan S, Zuriaga MA, Polackal MN, Ostriker AC, Chakraborty R, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*. 2017 Feb 24;**355**:842–847.
12. Ricard L, Hirsch P, Largeaud L, Deswarte C, Jachiet V, Mohty M, et al. Clonal haematopoiesis is increased in early onset in systemic sclerosis. *Rheumatology (Oxford)*. 2020 Aug 5;
13. Savola P, Lundgren S, Keränen MAI, Almusa H, Ellonen P, Leirisalo-Repo M, et al. Clonal hematopoiesis in patients with rheumatoid arthritis. *Blood Cancer J*. 2018 **26**;8:69.
14. Costedoat-Chalumeau N, Galicier L, Aumaître O, Francès C, Le Guern V, Lioté F, et al. Hydroxychloroquine in systemic lupus erythematosus: results of a French multicentre controlled trial (PLUS Study). *Ann Rheum Dis*. 2013 Nov;**72**:1786–1792.
15. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006 Feb;**4**:295–306.
16. Weening JJ. The Classification of Glomerulonephritis in Systemic Lupus Erythematosus Revisited. *Journal of the American Society of Nephrology*. 2004 Feb 1;**15**:241–250.

17. Duployez N, Demonchy J, Berthon C, Goutay J, Caplan M, Moreau A-S, et al. Clinico-Biological Features and Clonal Hematopoiesis in Patients with Severe COVID-19. *Cancers (Basel)*. 2020 Jul 21;**12**:e1992.
18. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;**4**:1073–1081.
19. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010 Apr;**7**:248–249.
20. Young AL, Challen GA, Birman BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat Commun*. 2016 **22**;7:12484.
21. Acuna-Hidalgo R, Sengul H, Steehouwer M, van de Vorst M, Vermeulen SH, Kiemeny LALM, et al. Ultra-sensitive Sequencing Identifies High Prevalence of Clonal Hematopoiesis-Associated Mutations throughout Adult Life. *Am J Hum Genet*. 2017 Jul 6;**101**:50–64.
22. Arends CM, Weiss M, Christen F, Eulenberg-Gustavus C, Rousselle A, Kettritz R, et al. Clonal hematopoiesis in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. *Haematologica*. 2020 Jun;**105**(6):e264–e267.
23. Lai C-H, Lai W-W, Chiou M-J, Lin W-C, Yang Y-J, Li C-Y, et al. Outcomes of percutaneous coronary intervention in patients with rheumatoid arthritis and systemic lupus erythematosus: an 11-year nationwide cohort study. *Ann Rheum Dis*. 2016 Jul;**75**(7):1350–1356.
24. Gerard A-L, Goulenok T, Bahuaud M, Francois C, Aucouturier P, Moins-Teisserenc H, et al. Serum IgG2 levels predict long-term protection following pneumococcal

- vaccination in systemic lupus erythematosus (SLE). *Vaccine*. 2020 Oct 14;**38**:6859–6863.
25. Sacre K, Goulenok T, Bahuaud M, Francois C, Van der Haegen MC, Alexandra J-F, et al. Impaired long-term immune protection following pneumococcal 13-valent/23-valent polysaccharide vaccine in systemic lupus erythematosus (SLE). *Ann Rheum Dis*. 2018 Oct;**77**:1540–1542.
26. Clement M, Charles N, Escoubet B, Guedj K, Chauveheid M-P, Caligiuri G, et al. CD4+CXCR3+ T cells and plasmacytoid dendritic cells drive accelerated atherosclerosis associated with systemic lupus erythematosus. *J Autoimmun*. 2015 Sep;**63**:59–67.
27. Fritsch RD, Shen X, Illei GG, Yarboro CH, Prussin C, Hathcock KS, et al. Abnormal differentiation of memory T cells in systemic lupus erythematosus. *Arthritis Rheum*. 2006 Jul;**54**:2184–2197.
28. Bick Alexander G., Pirruccello James P., Griffin Gabriel K., Gupta Namrata, Gabriel Stacey, Saleheen Danish, et al. Genetic Interleukin 6 Signaling Deficiency Attenuates Cardiovascular Risk in Clonal Hematopoiesis. *Circulation*. 2020 Jan 14;**141**:124–131.
29. Rovenský J, Tuchynová A. Systemic lupus erythematosus in the elderly. *Autoimmun Rev*. 2008 Jan;**7**:235–239.

FIGURE LEGENDS

Figure 1 Clonal hematopoiesis of indeterminate potential in SLE patients and controls according to age

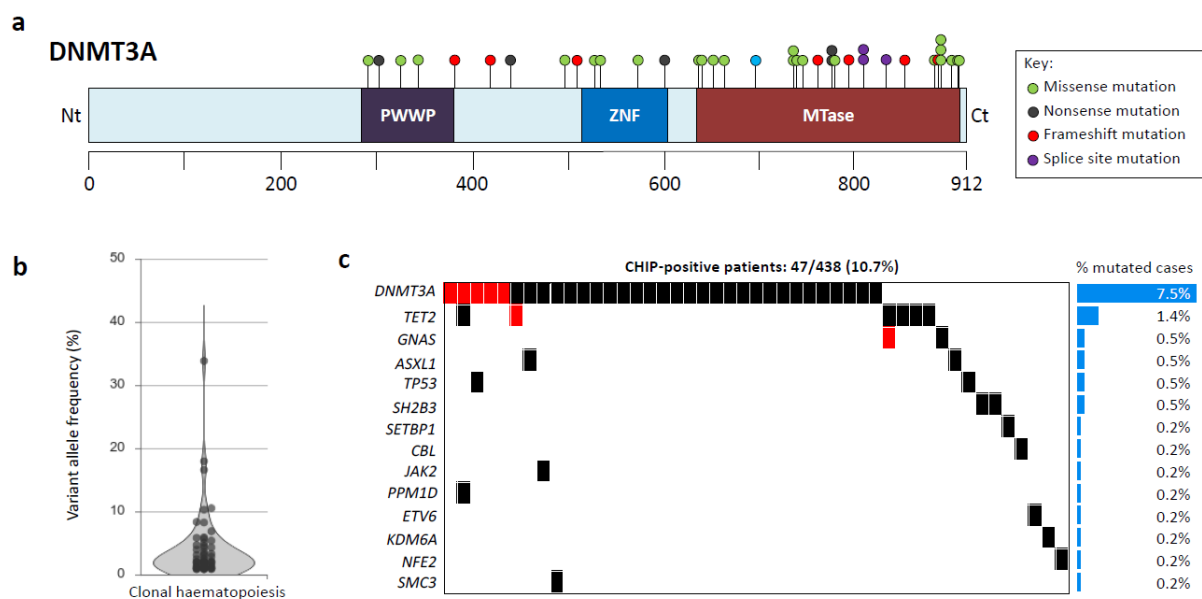


Number of patients with (black bars) or without (grey bars) CHIP is given with respect to age in SLE (a) and controls (b). Dotted line represents the median age of all (SLE or controls) patients and full line, the median age of (SLE or controls) patients with CHIP. Analysis was performed on 438 SLE patients and 479 controls. Prevalence of CHIP per age group (c), defined as the frequency of individuals with one or more CHIP per decade in SLE and controls. Analysis in controls was performed on 34 subjects for age group 30–39, 41 for age group 40–49, and 77 for age group 50–59. Analysis in SLE was performed on 132 patients for age group 30–39, 108 for age group 40–49, and 57 for age group 50–59.

CHIP prevalence in SLE and controls were assessed in a single laboratory by using the same pipelines (see Material and Methods).

CHIP, clonal hematopoiesis of indeterminate potential; CTL, controls

Figure 2 Mutations in genes associated with clonal hematopoiesis in SLE



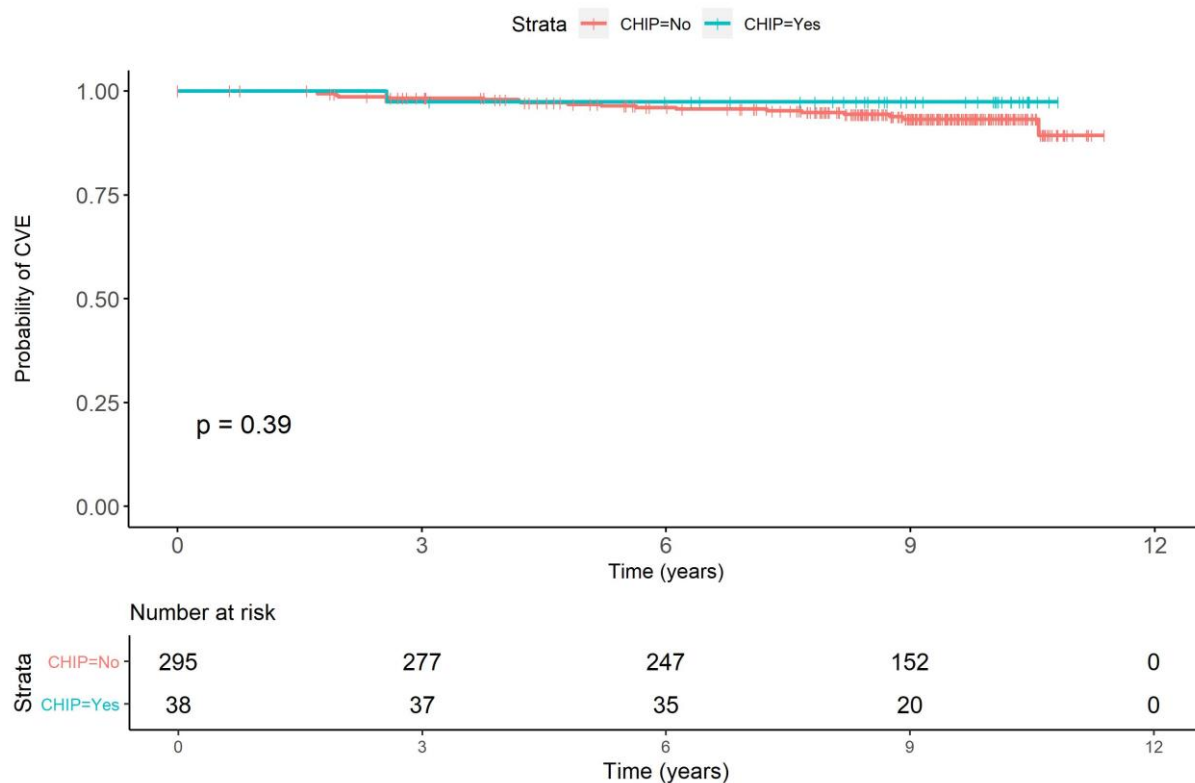
Lollipop plots (a) depicting *DNMT3A* mutations in SLE patients. Black, green, red, and purple dots indicate frameshift/nonsense, missense, in frame, and splicing mutations, respectively.

Violin plot (b) showing the distributions of variant allele frequencies of clonal hematopoiesis.

Molecular landscape (c) showing co-mutations in 47 clonal hematopoiesis-positive patients.

Red boxes indicate several mutations within the same gene.

Figure 3 Kaplan-Meier curves reflecting cumulative proportion of SLE patients free of CVE according to CHIP status at baseline



Analysis was performed on 333 patients

CVE: cardiovascular event; CHIP, clonal hematopoiesis of indeterminate potential

TABLES

Table 1 Characteristics of SLE patients at inclusion

| Characteristics | SLE Patients, n=438 |
|--|---------------------|
| Age, years | 38 [29-47] |
| Female gender, n (%) | 402 (91.8) |
| Smoker current, n (%) | 55 (12.6) |
| Diabetes, n (%) | 8 (1.8) |
| LDL-Cholesterol, mg/dL | 100 [84-122] |
| Dyslipidemia, n (%) | 23 (5.3) |
| Hypertension, n (%) | 13 (3) |
| BMI>30kg/m ² , n (%) | 45 (10.3) |
| eGFR ^a <60 ml/mn/1,73m ² , n (%) | 0 (0) |
| Duration of SLE disease, years | 8 [3-25-13] |
| Lupus nephritis, n (%) | 87 (19.9) |
| aPL antibodies, n (%) | 201 (45.9) |
| APS, n (%) | 50 (11.4) |
| SLEDAI score | 2 [0-2] |
| SLEDAI >4, n (%) | 48 (11) |
| Low C3, n (%) | 35 (8) |
| Hormonal contraception, n (%) | 152 (34.7) |
| Steroids, n (%) | 295 (67.4) |
| Steroids daily dose at inclusion, mg/d | 8 [5-10] |
| Hydroxychloroquine, n (%) | 433 (98.9) |
| Immunosuppressive drugs ^b , n (%) | 169 (38.6) |
| Biologics ^c , n (%) | 28 (6.4) |
| Antiplatelet treatment, n (%) | 105 (24) |
| Statin, n (%) | 19 (4.3) |
| ACE inhibitors, n (%) | 97 (22.1) |
| Anticoagulant treatment, n (%) | 44 (10) |

Results are shown as median [IQR] or number (percentage)

^aEstimated glomerular filtration rate (eGRF) was calculated with the Modification of Diet in Renal Disease (MDRD) equation

^bImmunosuppressive drugs included Azathioprine, Methotrexate, Cyclosporine, Mycophenolate mofetil, Cyclophosphamide, Thalidomide, Tacrolimus, Lenalidomide or Sulfasalazine.

^cBiologics included Rituximab or Belimumab.

ACE: angiotensin converting enzyme, AP: anti phospholipid, APS: anti phospholipid syndrome, BMI: body mass index, LDL: low-density lipoprotein, SLE: systemic lupus erythematosus, SLEDAI: systemic lupus erythematosus disease activity index.

Table 2 Cluster analysis of SLE patients

| | Total n=438 | Cluster 1 n=156 | Cluster 2 n=81 | Cluster 3 n=122 | Cluster 4 n=79 | p-value |
|------------------------------|----------------|--------------------|-------------------|--------------------|-------------------|---------|
| Age at inclusion, years | | | | | | <0.0001 |
| [18,33] | 154 (35.2) | 38 (24.4) | 28 (34.6) | 65 (53.3) | 23 (29.1) | |
| [33,44] | 147 (33.6) | 52 (33.3) | 24 (29.6) | 39 (32) | 32 (40.5) | |
| [44,82] | 137 (31.3) | 66 (42.3) | 29 (35.8) | 18 (14.8) | 24 (30.4) | |
| Disease duration, years | | | | | | <0.0001 |
| [0,5] | 163 (37.2) | 59 (37.8) | 36 (44.4) | 59 (48.4) | 9 (11.4) | |
| [5,11] | 149 (34) | 56 (35.9) | 20 (24.7) | 49 (40.2) | 24 (30.4) | |
| [11,38] | 126 (28.8) | 41 (26.3) | 25 (30.9) | 14 (11.5) | 46 (58.2) | |
| Ethnic group | | | | | | <0.0001 |
| European | 264 (60.3) | 126 (80.8) | 49 (60.5) | 39 (32) | 50 (63.3) | |
| North african | 61 (13.9) | 7 (4.5) | 20 (24.7) | 22 (18) | 12 (15.2) | |
| Afro-american | 69 (15.8) | 17 (10.9) | 2 (2.5) | 47 (38.5) | 3 (3.8) | |
| Asian | 36 (8.2) | 4 (2.6) | 7 (8.6) | 13 (10.7) | 12 (15.2) | |
| Other | 8 (1.8) | 2 (1.3) | 3 (3.7) | 1 (0.8) | 2 (2.5) | |
| Clinical characteristics | | | | | | |
| Autoimmune hemolysis | 52 (11.9) | 10 (6.4) | 15 (18.5) | 14 (11.5) | 13 (16.5) | 0.0236 |
| Leukopenia | 85 (19.4) | 29 (18.6) | 10 (12.3) | 29 (23.8) | 17 (21.5) | 0.2252 |
| Thrombocytopenia | 92 (21) | 16 (10.3) | 33 (40.7) | 19 (15.6) | 24 (30.4) | <0.0001 |
| Rash | 222 (50.7) | 70 (44.9) | 49 (60.5) | 44 (36.1) | 59 (74.7) | <0.0001 |
| Discoid lupus | 54 (12.3) | 25 (16) | 6 (7.4) | 15 (12.3) | 8 (10.1) | 0.2465 |
| Photosensitivity | 254 (58) | 118 (75.6) | 49 (60.5) | 39 (32) | 48 (60.8) | <0.0001 |
| Oral ulcers | 67 (15.3) | 17 (10.9) | 8 (9.9) | 20 (16.4) | 22 (27.8) | 0.0031 |
| Arthritis | 381 (87) | 146 (93.6) | 50 (61.7) | 114 (93.4) | 71 (89.9) | <0.0001 |
| Pleural/pericardial effusion | 110 (25.1) | 23 (14.7) | 24 (29.6) | 37 (30.3) | 26 (32.9) | 0.0028 |
| Delirium or psychosis | 30 (6.8) | 6 (3.8) | 7 (8.6) | 11 (9) | 6 (7.6) | 0.3105 |
| Proteinuria >0.5g/24h | 128 (29.2) | 5 (3.2) | 6 (7.4) | 39 (32) | 78 (98.7) | <0.0001 |
| Class III/IV/V nephritis | 89 (20.3) | 0 (0) | 2 (2.5) | 21 (17.2) | 66 (83.5) | <0.0001 |
| aPL | 201 (45.9) | 23 (14.7) | 70 (86.4) | 60 (49.2) | 48 (60.8) | <0.0001 |
| Low C3 | 71 (16.2) | 10 (6.4) | 6 (7.4) | 33 (27) | 22 (27.8) | <0.0001 |
| Low C4 | 82 (18.7) | 19 (12.2) | 6 (7.4) | 33 (27) | 24 (30.4) | <0.0001 |
| Anti-dsDNA antibodies | 385 (87.9) | 129 (82.7) | 63 (77.8) | 116 (95.1) | 77 (97.5) | <0.0001 |
| Anti-Sm antibodies | 76 (17.4) | 13 (8.3) | 3 (3.7) | 43 (35.2) | 17 (21.5) | <0.0001 |
| APS | 50 (11.4) | 0 (0) | 31 (38.3) | 3 (2.5) | 16 (20.3) | <0.0001 |
| CHIP | 47 (10.7) | 35 (22.4) | 1 (1.2) | 3 (2.5) | 8 (10.1) | <0.0001 |

aPL; antiphospholipid antibodies (lupus anticoagulant and/or anti-cardiolipin antibodies), APS: antiphospholipid syndrome, C: complement; CHIP: clonal hematopoiesis of indeterminate potential, dsDNA: double stranded DNA, Sm: Smith

Class III/IV/V nephritis based on International Society of Nephrology/Renal Pathology Society classification.¹⁶