A Longitudinal Multiethnic Study of Biomarkers in Systemic Lupus Erythematosus: Launching the GLADEL 2.0 Study Group

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Author contribution

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Abstract

After more than 20 years of sustained work, the Latin American Group for the Study of Lupus (GLADEL) has made a significant number of contributions to the field of lupus, not only in the differential role that race/ethnicity plays in its course and outcome but also in several other studies including the beneficial effects of using antimalarials in lupus patients and the development of consensus guidelines for the treatment of lupus in our region.

A new generation of "Lupus Investigators" in more than 40 centers throughout Latin America has been constituted in order to continue the legacy of the investigators of the original cohort and to launch a novel study of serum and urinary biomarkers in patients with systemic lupus erythematosus. So far, we have recruited 783 patients from 42 Latin-American centers including 334 patients with SLE without renal involvement, 198 patients with SLE with prevalent but inactive renal disease, 168 patients with prevalent and active renal disease and 83 patients with incident lupus nephritis.

The different methodological aspects of the GLADEL 2.0 cohort are discussed in this manuscript, including the challenges and difficulties of conducting such an ambitious project.

Keywords: Lupus, biomarkers, multiethnic, lupus nephritis, Latin American

Introduction

The Latin American Group for the Study of Lupus (GLADEL, for *Grupo Latino Americano De Estudio del Lupus*) was constituted in 1997 as a network of rheumatologists with especial interest in lupus and with the aim of studying its features, course and outcome in Latin-American patients. The original inception cohort enrolled 1480 patients with a recent diagnosis (≤ 2 years) of systemic lupus erythematosus (SLE), in 34 Latin American centers with expertise in lupus from nine Latin-American countries (1,2).

The original GLADEL cohort has made important contributions in the field of lupus (2) not only by providing data on the largest cohort of Mestizo (Caucasian and Amerindian ancestral background) SLE patients, but also in some other areas including familial aggregation (3), lupus in males and in childhood (4,5) and late onset lupus (6). Importantly, GLADEL has confirmed or described the benefits of antimalarials in several clinically significant aspects: preventing disease flares (7), preventing high disease activity early in the course of the disease (8), preventing renal (9,10), cardiac (11), and hematological disease involvement (12), protecting from serious infections (13) and delaying mortality (14). Also in favoring remission and low disease activity, among others (15,16).

More recently, GLADEL, as PANLAR's Lupus Study Group, elaborated evidence-based guidelines for the treatment of SLE using the GRADE methodology (17) and covering the most important lupus domains with a special interest in local problems and regional publications (18).

Milestones of GLADEL 2.0 cohort

In 2014, during the ACR/ARHP Annual Meeting held in Boston (https://www.rheumatology.org), a young group of rheumatologists from different Latin-American centers, with experience and interest in lupus, was incorporated to the original cadre of investigators; this new group was called GLADEL 2.0. This project envisioned the inclusion of young researchers (under 40 years of age), from different countries, trained in different lupus clinics around the world, to the Latin-American network for the study of lupus. During that meeting a brainstorming and a strategic planning session took place. More than 50 senior and junior lupus researchers from more than 10 Latin-American countries participated. Recognition of local needs, including a new GLADEL cohort and guidelines, as well as the identification of future members of this collaborative network took place. In September 2015, during the 11th International Congress on Systemic Lupus Erythematosus, held in Vienna, Austria (https://lupus2015.org), we identified the groups priorities and possibilities. As a result, the first group project on serum and urinary biomarkers in a new cohort of prevalent and incident SLE patients was agreed upon. In 2016, during the XIX PANLAR congress held in Panamá (https://www.panlar.org/xix-panlar-panama), the first presentation of the project to the scientific community took place. This forum provided the opportunity for the

participation of academic centers from the initial GLADEL cohort as well as new lupus centers from the region.

Given the continuous discovery of new biomarkers for the diagnosis and follow-up of SLE patients, we designed a longitudinal international collaborative study in Latin-American countries to better understand the usefulness, yield and validity of novel biomarkers in a large multinational, multicenter and multiethnic cohort.

Background and aims of the study

The search for new biomarkers in SLE has been the subject of interesting research including serological and urinary markers (19). Regarding serological biomarkers, during the last decade, several new autoimmune biomarkers have been developed. For example, anti-dense fine speckled (DFS70) antibodies have proved useful to differentiate patients positive for antinuclear antibodies (ANA) by immunofluorescence (IMF) but without a well-defined underlying disease from patients with an established autoimmune disease. Anti-DFS70 antibodies have demonstrated a negative association with ANA associated rheumatic diseases, especially when the antibodies do not occur in association with other clinically relevant autoantibodies (20). In a recent study, including 127 individuals with ANA positivity, the presence of anti-DFS70 autoantibodies was confirmed in 33% of healthy controls but in only 12.5% patients with SLE (21).

In antiphospholipid syndrome (APS), some novel markers have been reported recently (22). There is high variability in the prevalence of antiphospholipid antibodies (aPL) and isotype distribution in different populations of patients with primary APS and SLE (23). In the original GLADEL cohort (1), with almost half of the patients tested for aPL, African-Latin Americans showed the lowest rate of lupus anticoagulant (LA) and IgG and IgM anticardiolipin antibodies (aCL) when compared to Caucasian and Mestizo patients (1). Because the GLADEL original cohort was launched in 1997 when only conventional biological aPL markers were available (LA and aCL), numerous other markers of APS have been studied since, such as antibodies against β2-glycoprotein-I (anti-β_2GP1), and phosphatidylserine/prothrombin (anti-PS/PT), among others. These may be helpful to establish lupus clusters as well as for the study in SLE of the so-called seronegative APS, which defines a group of patients with clinical manifestations of APS but with persistently negative aCL, β_2GP1 antibodies and LA (22).

Another promising area is the field of urinary biomarkers in SLE. Currently, only anti-double stranded DNA antibodies (anti-dsDNA) and serum complement are non-invasive biomarkers for monitoring renal activity in patients with lupus nephritis (LN) in the clinical setting. However, these markers reflect immunological activity but are not markers of kidney inflammation or damage (24).

Several urinary biomarkers have been described in patients with SLE and specifically in patients with LN. These markers in many cases are not specific for SLE, but rather reflect a series of cellular processes both at the glomerular and tubular levels, and may be due to inflammatory, fibrotic or ischemic changes in the renal structures (19). Within the large

group of biomarkers, studies related to cytokines (IL-17, IL-22), chemokines (MCP-1, IL-8), adipokines (resistin and leptin), and various proteins including the TNF superfamily cytokine TWEAK, lipocalin associated with neutrophil gelatinase (NGAL), transferrin, ceruloplasmin and osteoprotegerin (OPG) among others (19,23,25,26), have been published.

Recently in a Colombian SLE population, both MCP-1 and NGAL were identified as useful markers to discriminate patients with or without LN. Also, urinary NGAL and MCP-1 were significantly higher in patients with active LN than in inactive LN (27). Similarly, in another study, urinary levels of ceruloplasmin and transferrin were higher in patients with LN compared to those without LN and in patients with active LN compared to those with inactive LN (28).

Urinary TWEAK (uTWEAK) has also shown promising results in recently diagnosed Mexican SLE patients. Active LN patients had higher levels of uTWEAK than patients with other glomerulopathies and healthy controls (29). uTWEAK had also been related to class-V LN (30), and more recently as a marker of higher risk of developing end-stage renal disease (31).

Despite these promising data, these results have some limitations. Similar results have not been replicated in all populations and most studies have been cross-sectionally or with a relatively short follow-up time. For all of the mentioned reasons, more studies with larger multi-ethnic cohorts and long-term follow-up are required. Our aim is to identify subgroups of patients with SLE, not only based on demographic and clinical profile, but also based on serum and urinary biomarkers and transcriptome studies using blood and tissue RNA to identify potential transcriptional signatures.

Center selection

The 42 centers participating in this new GLADEL cohort are distributed among 10 Latin-American countries (Argentina, Brazil, Chile, Colombia, Dominican Republic, Ecuador, México, Paraguay, Perú and Uruguay). To be included, the centers had to meet the following criteria: have experience in SLE (referral centers with a lupus clinic, an academic profile, and a rheumatology training program); have a genuine interest in the research project; and have an identified leader, as well as adequate human, technical, and communication facilities.

Inclusion criteria

We are including consecutive patients with SLE according to one of the classification criteria: 1982/1997 American College of Rheumatology (ACR) (32,33) and/or 2012 Systemic Lupus International Collaborating Clinics (SLICC) (34), age ≥ 18 years, who agree to participate and sign the corresponding informed consent. Patients with APS associated with lupus could also be included. We are using those former classification criteria because our study was initiated before definition and publication of current classification criteria for SLE (ACR/EULAR 2019 criteria) (35). Since we are collecting a wide number of

clinical and serological data, we are confident to perform analysis in the future based on new criteria. All patients with lupus will have a healthy non-relative control at ratio 1:1, matched by age (± 5 years), sex and ethnic group. These controls should not have any of the following: autoimmune disorders, diabetes, hypertension or other renal or systemic disease that might compromise the objectives of the study according to the local investigator's criteria.

Exclusion criteria

Patients with other systemic autoimmune diseases or overlapping syndromes (rheumatoid arthritis, systemic sclerosis, dermato-polymyositis, systemic vasculitis and others), pregnancy, active urinary or systemic infection at the time of inclusion, hepatitis B, C or human immunodeficiency virus-positivity will be excluded from the study. Patients with concomitant Sjogren's syndrome and antiphospholipid syndrome will not be excluded.

Patient selection

Each center will include 25 patients with SLE divided into four groups: Group 1: patients with SLE, without renal involvement (never), of any disease duration (total: 10 patients); Group 2: patients with SLE, with prevalent renal involvement (at any time during their disease course), currently inactive (total: 5 patients); Group 3: patients with SLE, with prevalent renal involvement (at any time during their disease course), currently active (total: 5 patients); and Group 4: patients with SLE, with incident renal involvement (maximum three months), with renal biopsy (mandatory criterion), and prioritizing those patients who either have not received immunosuppressive treatment, or who have not completed the first cycle of induction treatment for remission of LN (total: 5 patients).

Study design

Our study includes a cross-sectional phase where serum and urinary markers will be evaluated in both SLE patients and healthy controls. Each center will recruit 25 SLE patients divided into the aforementioned groups. Additionally, serial measurements of biomarkers in centralized labs will be performed at least at baseline, and at 6 and 12 months of follow-up. Yearly follow-up visits will take place at least until year five. Likewise, transcriptomic studies in both whole blood and tissues will be carried out in a selected group of patients. The study design is summarized in Figure 1.

Study variables

The study protocol includes sociodemographic variables (gender, ethnicity, marital status, socioeconomic level, level of education, medical coverage and occupation), physical examination and habits, accumulated lupus criteria, clinical manifestations (general, skin and mucosa, musculoskeletal, renal, renal biopsies, neuro-psychiatric, ocular, serositis, cardiovascular, pulmonary, gastrointestinal, hematological and maternal-fetal

manifestations, infections, hospitalizations, and comorbidities), personal and family history of other autoimmune diseases, treatment schemes (specific for lupus manifestations and general treatments for other situations and comorbidities), local laboratories (hematological, chemical, urine analysis and autoantibodies and complement), the Physician Global Assessment (PGA), disease activity by the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (36) and disease damage by the Systemic Lupus International Collaborating Clinics American College of Rheumatology Damage index (SLICC/ACR DI) (37).

Patient-reported outcomes

In addition, two patient-reported outcomes (PROs) will be included: the Lupus Quality of Life (LupusQoL) (38) and the Work Productivity and Activity Impairment questionnaire (WPAI: Lupus) (39).

Serum markers

We are planning to determine several serological biomarkers in the SLE patients as well as in the healthy controls. These biomarkers will be performed at the local laboratory of each of the centers and, in parallel, at the centralized laboratories, which will allow comparative studies to be carried out. Serum samples will be stored at−70◦C before shipment to central labs. It is important to emphasize that for the first time in a Latin-American SLE multicenter study, these techniques will be performed by core laboratories, with the unequivocal benefit derived from examining these biomarkers centrally.

Serum biomarkers will include some conventional tests such as ANA by IMF assay, anti-dsDNA, aCL IgG and IgM as well as IgG and IgM anti-β_2GP1. The panel of novel autoantibodies include at least anti-C1q antibodies as well as aPS/PT IgG and IgM antibodies by ELISA techniques. Anti-DFS-70 antibodies in SLE and healthy individuals will be measured by chemiluminescence. For conventional SLE antibodies more stable over time (i.e. anti-Ro, anti-La, anti-Sm, anti-RNP and LA), results obtained previously at each center will be considered valid.

Urinary markers

Several biomarkers, but not the only ones, will be tested in the study including TWEAK, OPG, MCP-1 and RANTES. We will use commercial ELISA kits (Merck-Millipore). Briefly, urinary sample will be taken from each patient and healthy control in the morning, centrifuged, filtered, and finally they will be stored at−70◦C until the samples are analyzed. For the processing of the samples the instructions recommended by the manufacturer will be followed. Urinary biomarkers will only be performed at a centralized laboratory.

Transcriptome studies

Blood and tissue transcriptional studies will be performed at a central laboratory to potentially identify signatures associated with the aforementioned study variables. Transcriptome tissue analysis will be done either in kidney or skin samples in SLE patients and in skin tissue in the healthy controls.

Database

Data will be collected using ARTHROS-Web (www.arthrosoft.com), a modified version of the ARTHROS software. This is an already validated, user-friendly database developed by Argentine rheumatologists (40). Video tutorials on the management of ARTHROS are already available on YouTube (https://www.youtube.com/watch?v=T47BHU0557U). The data for the patients and healthy controls can only be modified by the investigators at the center loading them. The identity of the patients is confidential and all data are exported completely anonymized.

Data Control Committee

The project establishes the creation of a data control committee (DCC) to ensure the correct loading of information and avoid potential data loss. Once the data from the first patient and the corresponding healthy control (visit T0) is uploaded to ARTHROS-Web, each center must send an e-mail to the DCC so the information can be verified and corrections/adjustments requested, if necessary. To this end, the DCC will communicate this information to the centers in a personalized manner. The DCC will also be available to answer questions that may arise at each of the centers.

Ethical Considerations

This study is being conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonization (ICH) and by the ethical principles underlying the European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50).

The study is being conducted in compliance with the protocol. The protocol, any amendments, and the subject informed consent have received Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval/favorable opinion before initiation of the study at each of the 42 centers. Confidentiality of all participants will be maintained.

Initial steps and challenges

At August 9th, 2020, we have recruited 783 patients from 42 Latin-American centers. Our study is currently active in 10 countries with inclusion of all four lupus groups including 201 patients from Argentina, 149 from Brazil, 136 from México, 75 from Colombia, 66 from Perú, 48 from Uruguay, 41 from Paraguay, 29 from Chile, 18 From Ecuador and 17 from the Dominican Republic (Figure 2).

Included patients are distributed as follow: 334 belong to group 1, 198 belong to group 2, 168 belong to group 3 and 83 to group 4.

As expected, given the complex nature of our study, we are facing challenges and complications related to logistics. First, we had to deal with a wide range of regulatory committees in different countries as well as logistic aspects related to the distribution of samples to guarantee a homogeneous methodology. Second, as has been occurring in the rest of the world, at the end of March 2020, all Latin-American countries went to a preventive lockdown to mitigate the spread of the COVID-19 pandemic. Despite all of these challenges, our study remains active, recruiting patients and recording clinical data of the first follow-up of the patients. Some parts of our initial design may have to be modified according with new restrictions as a result of the pandemic. We are going to capitalize on this global scale disaster to assess coronavirus infection in our registry and to collaborate with the Global Alliance COVID19 (https://rheum-covid.org).

GLADEL 2.0: A glance into the future

The last 5-6 years have involved a significant number of work meetings, changes in the protocol design and efforts that have allowed us to gather a large number of centers throughout Latin-American which will allow us to generate a longitudinal cohort of lupus patients with high-quality standards and reliability in the information thanks to various internal project's control mechanisms.

Not without difficulties, we have so far included a large number of patients and healthy controls and we have overcome the limitations of a study of this nature with 10 countries and more than 40 active centers.

We are very enthusiastic because the present study covers several important areas of lupus which may improve our current knowledge. It will further examine ethnic and sociodemographic aspects which we already know are prognostic factors with a significant impact on the disease. It will also examine newer serum and urinary biomarkers as well as tissue studies that may further contribute to our understanding of organ involvement, particularly LN. Finally, it will also evaluate the impact on the quality of life and productivity of patients, thus covering a more comprehensive and holistic approach to the disease. The challenge has been great, but we hope that the benefits will be even greater, both for the scientific community and more importantly, for our patients.

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Figure 1. GLADEL 2.0 Cohort. Study design.

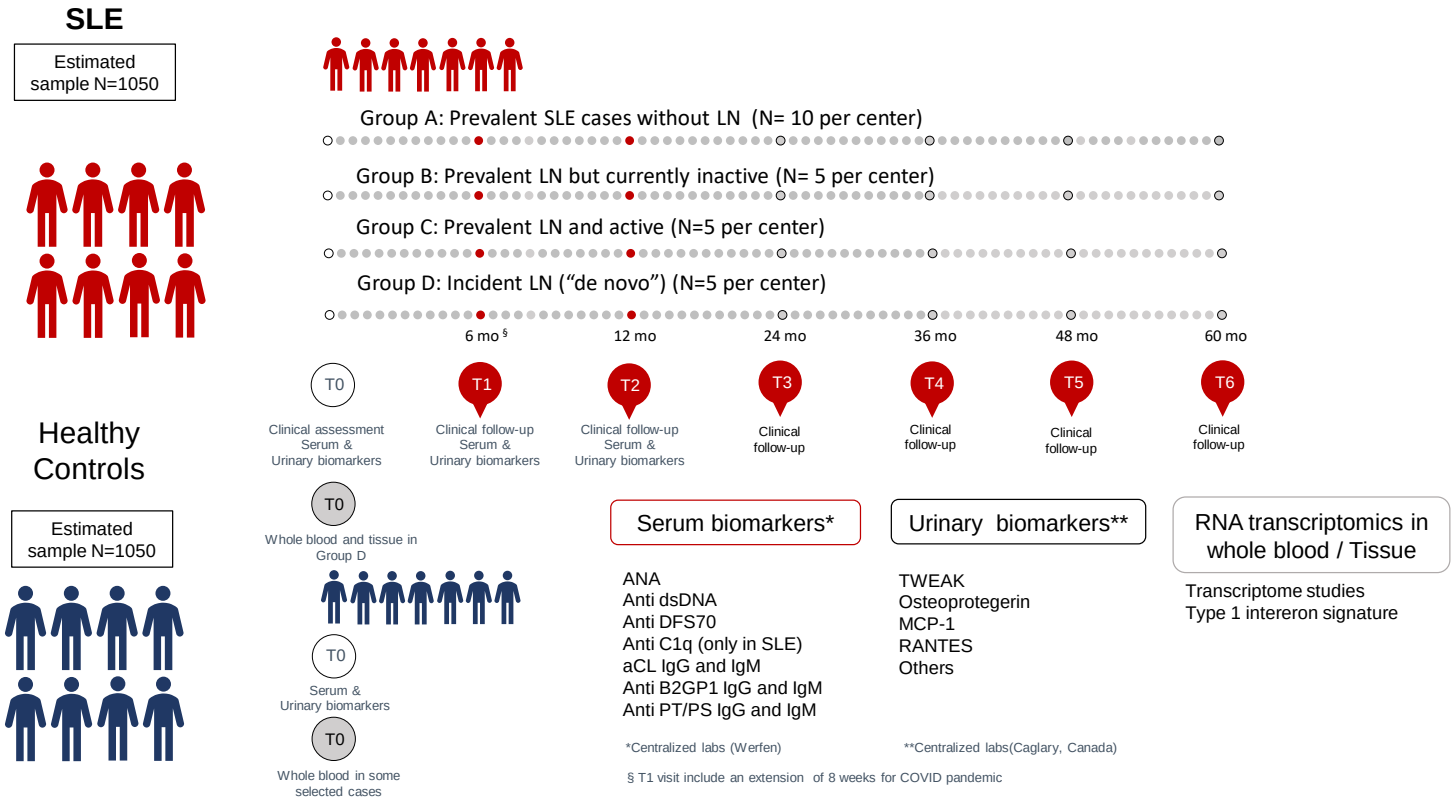

Figure 2. Map of Active Participants in GLADEL 2.0 Study