Project plan

The prevalence of IgG, IgM, and IgA antibodies against cardiolipin, β2-glycoprotein I and domain I of β2-glycoprotein I in a large cohort of patients with Systemic Lupus Erythematosus and comparison of the profile of this antiphospholipid serology between those patients with juvenile versus adult onset disease.

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INTRODUCTION/BACKGROUND

The antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterised by vascular (arterial and/or venous) thrombosis (VT) and/or pregnancy morbidity (PM), associated with the presence of a specific group of autoantibodies called antiphospholipid antibodies (aPL) [1,2]. In the current international classification criteria for the APS three are the tests which define the laboratory criteria [3]. Two enzyme-linked immunosorbent assays (ELISAs) to measure anti-cardiolipin (aCL) and anti-β2-glycoprotein I (aβ2GPI) antibodies, of IgG and/or IgM isotype, and one third test which is a functional clotting assay for lupus anticoagulant (LA) [3]. However the presence of aPL is not limited to APS patients but they can be detected in several different settings, such as systemic autoimmune diseases, infectious conditions, cancer, use of particular drugs, and even in healthy individuals [4,5,6,7,8].

APS which is related to other autoimmune systemic diseases is known as secondary APS [9]. Most commonly secondary APS occurs in the spectrum of the clinical manifestations of Systemic Lupus Erythematosus (SLE) [2,9]. According to a European study of 1000 patients 36% of the cases of APS were associated with SLE [2]. However as many SLE patients are asymptomatic aPL carriers, there is need for new laboratory tests for APS which offer better prognostic value in these patients. They have been proposed as new tests ELISAs that measure IgA aPL and autoantibodies against Domain I of β2GPI (DI), which is the first out of the five domains of β2GPI [10,11]. IgA anti-β2GPI positivity is associated with both VT and PM in patients negative to the current classification tests [12]. On the other hand IgG anti-DI antibodies (aDI) are most closely linked to APS, despite that it has also been reported the presence of antibodies against all other individual domains of β2GPI [13,14].

There are different studies regarding the prevalence of aPL in SLE, both in populations of pediatric and adult patients [15,16,17]. However an extensive profiling of antiphospholipid serology, including the “classical” IgG and IgM aPL, IgA aCL and aβ2GPI, IgG, IgM and IgA aDI, across all the age groups of lupus has not been done before to our knowledge.

PURPOSE

To estimate the prevalence of nine different aPL (aCL, aβ2GPI and aDI, each one in all three isotypes IgG, IgM and IgA) in a large lupus cohort with patients across all age groups. To compare this prevalence between juvenile SLE patients, adult SLE patients of juvenile onset disease and adult SLE patients of adult onset disease.

SCIENTIFIC QUESTIONS

How the prevalence of the nine different aPL varies among the different age groups of patients with SLE?
Does the level of the titer of each of the nine aPL correlates with the duration of the SLE disease?

Which of the nine aPL were positive in SLE patients with clinical manifestations of APS? What was the prevalence of the respective aPL in this population?

Are aPL of IgA isotype and/or aDI useful in the diagnosis and/or prognosis of APS in lupus patients?

Is the presence of any of the nine aPL associated with specific clinical manifestations of SLE, i.e. kidney or CNS involvement?

**MATERIALS/METHODS**

The project is performed with the use of serum samples from patients within the SLE cohort of the Department of Rheumatology, University College London (UCL). The relevant demographics (age, gender, ethnicity) as well as the clinical data (SLE manifestations/organ involvement, presence or not of APS related manifestations, autoimmune lupus serology, treatment data) are provided.

The ELISA procedure for the detection of the respective aPL is as follows:

Half of a 96 well-plate is coated with antigen while the other half is treated with buffer alone. By subtraction of the optical density (OD) of the non-coated half from the OD of the antigen-coated half is obtained the net OD. Depending on which antibody isotype is being measured the following horseradish peroxidase conjugates are used: IgG – A6029, Sigma UK; IgM – A6927, Sigma UK; IgA – ab97215, Abcam UK. Serum is tested in duplicate at 1:50 dilution and in all nine assays intra-plate variation is < 10%. For the measurement of aCL are being used commercial calibrators (Louisville APL Diagnostics, TX, USA) and is being followed per consensus criteria protocols as previously described [18,19]. Activity is defined as IgG/IgM/IgA phospholipid units: respectively GPLU/MPLU/APLU. The calibrator’s activity ranges are 16-96 GPLU; 16-96 MPLU; and 2.7-120 APLU. aβ2GPI and aDI activity is also measured as previously described [19]. However regarding aDI, instead of human β2GPI, plates are coated with bacterially-expressed human recombinant DI [20]. Also, for both aβ2GPI and aDI assays, in-house calibrators are being used. These calibrators are obtained from the serum of patients with APS and high titers of the respective aPL, after serial dilution to obtain a standard curve followed by the assignation of arbitrary activity units to each point of the curve. aβ2GPI and aDI activity are defined as IgG/IgM/IgA aβ2GPI units (GBU/MBU/ABU respectively) and DI units (GDIU/MDIU/ADIU respectively). For the aβ2GPI assays the calibrator’s activity ranges are 3-100 GBU; 13-100 MBU; 7-100 ABU and for the aDI assays the ranges are 3-100 GDIU; 9-100 MDIU; 2-100 ADIU.

After completion of all experiments the prevalence of each of the nine aPL is reported as percentage of the total number of the project’s patients with SLE (currently n=283). For each patient with SLE the disease duration in years is calculated by subtraction of the patient’s age at diagnosis from the age when the serum sample was taken. Correlation and logistic regression analyses as well as receiver operating characteristic analysis (ROC) are to be performed using STATA to respond to the
scientific questions to be answered. It will be reported where appropriate, P values which determine significant negative or positive associations, odds ratios (OR) and 95% confidence intervals (95% CI).

ETHICAL CONSIDERATIONS

Application to an ethic committee has not been planned and is not necessary prior to the initiation of the project. An informed consent, approved by local research ethic committees, has already obtained from each patient prior to their inclusion to the SLE cohort.

SCHEDULE AND IMPLEMENTATION

During autumn 2014 I attended the Centre for Rheumatology and Bloomsbury Rheumatology Unit at the Rayne Institute of the University College London (UCL) where I trained in the research laboratory and introduced to the ELISA technique. I initiated the project by performing the nine different ELISA protocols on 73 of the 168 juvenile onset SLE samples. Other members of the research group will continue performing the experiments to the remaining samples. For 115 adult patients with SLE of adult onset, results for the nine ELISAs obtained from experiments during a previous project of the group. All the necessary demographics and clinical data have been collected during the time I was in London. After completion of all the experiments I have been set responsible to analyze the results using the required statistical methodology. Research at UCL is funded by grants from Arthritis Research UK.

IMPORTANCE

This project is the first one to compare IgG, IgM and IgA aCL, aβ2GPI and aDI in such an extensive age range of patients with SLE, with or without APS. Better markers are needed to distinguish those patients with SLE at risk of developing APS. Potentially, such an extensive APS serology with nine different aPL, can help in an attempt to identify these patients at risk who can benefit from prophylactic anticoagulation. Furthermore, if it can be demonstrated that the longer the duration of the SLE, the higher the prevalence and the titer of aPL, then awareness from clinicians regarding APS risk can focus in patients with longstanding disease. Also it would be useful to know if the presence of any of the nine aPL, or perhaps a combination of them, is more prevalent in patients with more aggressive lupus, as then aPL can potentially add extra value to the current diagnostic armamentarium for evaluation of lupus.

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