On cerebral lupus: from pathogenesis to clinical outcomes

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SUMMARY AND CONCLUSIONS

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SUMMARY, CONCLUSIONS AND FUTURE PERSPECTIVES

The studies included in this thesis focus in different aspects of the pathogenesis, diagnosis and outcome of neuropsychiatric (NP) manifestations presenting in patients with systemic lupus erythematosus (SLE). In particular the following topics were addressed. First, laboratory biomarkers, specifically serum complement cascade and autoantibodies, and their associations with NP-SLE manifestations and pathophysiological changes as seen on magnetic resonance imaging (MRI) were analyzed. Second, the role of quantitative neuroimaging techniques in the detection of brain microstructural changes and in the identification of underlying pathophysiological process in NP-SLE was assessed. Third, the value of multidisciplinary re-assessment of patients in the attribution of NP manifestations to SLE or other etiologies was evaluated and its role as gold standard in the diagnostic process of NP-SLE was discussed. Finally, we investigated if the clinical and patient´s related outcomes of NP manifestations presenting in SLE were associated with a different underlying pathophysiological process.

This final chapter summarizes the main findings of the studies comprised in this thesis and suggests potential future research paths to address important unmet needs in the NP-SLE field. The factors that need to be addressed to improve the diagnostic or attribution process of NP manifestations presenting in SLE patients and the extent to which new therapies may impact the future treatment of NP-SLE will also be discussed.

Summary and conclusions

Laboratory biomarkers

To start, in **Chapter 2**, we presented the story of a case of C1q deficiency associated with severe inflammatory and ischemic NP-SLE. C1q deficiency, a rare immunodeficiency, is probably the strongest susceptibility factor for the development of SLE and so far the only deficiency of early components of the complement classical pathway (C1q/C1r/C1s, C2, or C4) where NP involvement is present (20%).(1) A mutation in the C1qC-gene can either lead to complete deficiency or to low C1q levels with C1q polypeptide in the form of low-molecular weight (LMW) C1q. We showed how the serum of our patient contained very low levels of a non-functional LMW variant of C1q due to a homozygous G34R mutation in the C1qC-gene. We also provided a literature overview of NP-SLE in C1q deficiency and showed how these patients present with more severe forms of NP-SLE, mainly presenting with seizures and neuroimaging changes in basal ganglia and cerebral vasculitis, than in complement competent NP-SLE patients. We hypothesized about the potential role of C1q in the genesis

of nervous involvement in SLE and the subsequent presentation of NP-SLE manifestations. Therefore, we concluded that the classical pathway is not necessary to develop NP-SLE; however the absence of C1q and, subsequently, some of its biological functions may be associated with NP-SLE and a more severe presentation.

Decreased levels of complement components, complement activation and higher levels of antibodies against C1q (anti-C1q) are a hallmark of active SLE.(2) In **Chapter 3** we studied the relationships between serum levels of anti-C1q, C1q circulating immune complexes (CIC), complement activation and complement components in SLE patients during the first NP-SLE manifestation. We showed an association between focal NP-SLE and a decreased C4 and between diffuse NP-SLE and markedly decreased activation of the alternative pathway (AP50), a decreased C3 and higher levels of anti-C1q antibodies. Posterior multivariate analysis showed that these associations may be explained due to other factors such as antiphospholipid antibodies (aPL) in the case of focal NP-SLE and global disease activity in the case of diffuse NP-SLE. Importantly, among the individual NP-SLE syndromes, AP50 and C3 were markedly decreased in lupus psychosis and cognitive dysfunction, which warrants further research.

The association between serum antibodies and NP-SLE manifestations was analyzed in **Chapter 4**. As a novelty we examined autoantibody clusters and we used an addressable laser bead immunoassay test for the detection of multiple SLE specific autoantibodies. Four separate clusters of autoantibody profiles were identified: Cluster 1 no specific autoantibodies, Cluster 2 anti-dsDNA/anti-SSA/anti-SSB/anti-TRIM21, Cluster 3 anti-Sm/RNP and Cluster 4 anti-dsDNA/lupus anticoagulant (LAC)/anticardiolipin (aCL) IgM/IgG.

In our present study we found an association between Cluster 4 and NP-SLE, which was consistent with available literature.(3, 4) This association was especially important in major focal NP-SLE manifestations (cerebrovascular disease, chorea, seizures and myelopathy) and was stronger when patients with minor NP-SLE syndromes (headache, anxiety, cognitive dysfunction, and mild forms of depression) were excluded. An association between other autoantibodies analyzed with the microarray kit or clusters of these autoantibodies and NP-SLE manifestations were not found.

Neuroimaging biomarkers

There is an imperative need of finding radiological techniques that help highlighting a certain underlying pathogenic processes (ischemic or inflammatory) and subsequently guide therapy in NP-SLE. In **Chapters 5-7** we investigated whether the pathophysiological changes as seen on neuroimaging are associated to underlying immune abnormalities in SLE and whether these techniques are helpful to identify different subsets of NP-SLE.

The association between serum autoantibodies with specific brain-MRI abnormalities was analyzed in **Chapter 5**. Furthermore, we studied whether these structural changes were associated with other SLE-related or classical cardiovascular disease (CVD) risk factors. Serum autoantibodies tested were LAC, aCL IgG and IgM, anti-dsDNA, anti-SSA/Ro-52, anti-SSB/La, anti-Sm, anti-RNP and thereafter assessed individually and in groups (total number of autoantibodies). We demonstrated the lack of association between the total number and individual SLE-related autoantibodies with inflammatory-like lesions while the total number of antiphospholipid antibodies, especially the positivity for LAC, were associated with several ischemic brain abnormalities and cerebral atrophy. Furthermore, cumulative SLE-organ damage and modifiable CVD risk factors, such as hypertension, contribute to these ischemic changes pointing out the importance of systemic accelerated atherosclerosis in SLE. In order to reassure our results, the effect of the clinical neuropsychiatric status and a sensitivity analysis including Beta-2-Glycoprotein 1 antibodies IgG and IgM in the analysis were performed.

In **Chapter 6**, we assessed magnetic transfer imaging (MTI) in a prospectively followed cohort of SLE patients presenting NP symptoms either related or unrelated to SLE, to investigate whether these parameters may highlight different pathogenic NP-SLE processes (inflammatory or ischemic. Among the MTI parameters, previous research in small groups of NP-SLE patients demonstrated that magnetization transfer ratio (MTR) histogram peak height (HPH) can be used as a quantitative estimate of tissue microstructural integrity in the brain. (5, 6) We have applied MTR-HPHs in the white matter and grey matter of different NP-SLE subgroups including healthy controls, SLE, non-SLE related NP symptoms and NP-SLE. The last group was also divided into inflammatory NP-SLE and ischemic NP-SLE according to the suspected pathogenic mechanism. We demonstrated for the first time that white matter MTR-HPHs might provide evidence for the presence of inflammatory NP-SLE. We found that inflammatory NP-SLE patients have lower white matter MTR-HPH values when compared with ischemic NP-SLE, SLE patients without ever NP symptoms, non-SLE related NP symptoms and healthy controls. Moreover, in a prospective level and as previously suggested,(7) we confirmed how white matter MTR-HPH is sensitive to clinical changes, highlighting its potential role as radiologic biomarker in the diagnostic process and follow-up of NP-SLE patients and with the monitoring of future treatment trials.

Cell-specific microstructural alterations in the brain of SLE patients with and without history of NP-SLE were investigated in **Chapter 7**. To this aim we used a 7-T MRI scanner to acquire T1-weighted images, diffusion tensor imaging (DTI) datasets, and single volume diffusion-weighted magnetic resonance spectroscopy (DW-MRS) data from the anterior body of the corpus callosum. We showed how intracellular alterations and particularly changes in glia, as shown by an increase in the average diffusivities of total choline and total creatine,

significantly correlated with past NP-SLE and SLE activity. We suggested that diffusion properties of choline compounds and of total creatine are potentially unique markers for glial reactivity in response to inflammation and remarked the great potential of DW-MRS for the study of the aetiology of disease related changes in tissue microstructure of patients with SLE/NP-SLE.

Improving attribution of neuropsychiatric manifestations in SLE

The correct attribution of NP events to systemic lupus erythematosus or to an alternative etiology remains a challenge. Besides being a crucial issue, with important implications for management and prognosis of these patients, studies analyzing rigorously the attribution of NP events to SLE are very scarce. Several attribution models for NP events occurring in SLE have been proposed; however multidisciplinary expert physician judgment based on clinical and complementary tests remains the most reliable reference standard for NP-SLE diagnosis.(8, 9) The contribution of reassessment in the attribution process of NP events to SLE or other etiologies was addressed in **Chapter 8.** We showed how in clinical practice NP events presenting in SLE are too often attributed to an immune mediated origin and how re-assessment increases diagnostic accuracy in NP-SLE. Moreover, according to our data clinical judgment cannot be substituted by any of the current attribution models available so far. We showed how, until we find more reliable tests, clinical follow-up and re-assessment of these patients will remain as reference standard in NP-SLE diagnosis.

The clinical outcome of NP events presenting in SLE has been poorly studied. In **Chapter 9**, we analyzed in detail the clinical outcome and change in health-related quality of life (HRQoL), measured by the 36-item Short Form Health Survey (SF-36), of NP events either related and non-related to SLE and whether the different pathophysiological NP-SLE mechanisms (inflammatory or ischemic) had an impact on these outcomes. We showed that inflammatory NP-SLE events have a better clinical outcome and a meaningful improvement in SF-36 mental component summary score than non-NP-SLE and ischemic NP-SLE events. Importantly, SLE disease activity was key as predictor of these results. Our results reflect the reversibility of brain inflammation and the improvement of disease activity after starting immunosuppression. Therefore, we proposed that these outcomes are helpful as measurements in the follow-up of NP-SLE patients and for monitoring future therapy NP-SLE trials.

Challenges and future perspectives in the diagnosis of NP-SLE

Yet despite years of efforts of the NP-SLE scientific community, the number of clinically useful biomarkers and even of validated biomarkers is embarrassingly modest. A series of scientific challenges in the field have yet to be overcome:

Laboratory biomarkers

- *Identification of new neuronal surface antigens* responsible for NP-SLE. The antigen identification paradigm which has been successfully used with limbic encephalitis may be applied on NP-SLE to recognize unknown neuronal cell-surface protein(s).(10) Determination of neuronal immunoreactivity in different areas of brain and cerebellum of homogeneous clinical and radiological NP-SLE groups may be analyze and afterwards correlated with clinical symptoms and MRI characteristics. To identify the target antigen cultured neurons and mass spectrometry should be used. Lastly, brains of knock out animal models or cells deprived of the suspect antigen by siRNA knock down may confirm the specificity of these candidate autoantibodies.(11)

- *Complement cascade and IFN*-α: the exciting area of research in NP-SLE mice models on complement cascade and IFN-α need to be translated to human NP-SLE. The study of these two biomarkers may lead to a better understanding of pathogenic underlying mechanisms of synapse loss and will probably open the door to the use of new therapeutic strategies in NP-SLE.

- *Blood brain barrier (BBB):* The BBB is a network of endothelial cells and pericyte and astrocyte projections that regulates the entry of soluble molecules and cells into the brain parenchyma. It has been proposed that a disruption of the integrity of the BBB may have a potential pathogenic role in NP-SLE since this may permit the influx of neuropathic antibodies across the BBB. Brain tissue-reactive antibodies in NP-SLE are thought to be synthesized in the CNS, but also in peripheral organs (lymph nodes and bone marrow). In the last case it was proposed that these autoantibodies must pass through the BBB of SLE patients to exert an effect upon neurons. Although an important role of BBB has been supposed, we need better understand the BBB in human NP-SLE and the factors disrupting this barrier. Studies comparing serum and CSF and using quotients are warranted.

Neuroimaging biomarkers

- *Another look at conventional MRI (cMRI) – the case for more sophisticated characterization of lesions*: In recent years, characterization of lesions in other neurological disorders mimicking NP-SLE has advanced far beyond the basic lesion count or lesion load. A notable example is the work related to lesions and their pathological classification in multiple sclerosis (MS), a well-known mimicker of NP-SLE. The latest and most significant step in characterization of white matter lesions in MS came after examining the spatial relationship between lesions and large veins in a visualization method that superimposes FLAIR images containing lesion spatial information, and T_2^*-weighted images, showing vein distribution in great detail.(12) It was found that concentric co-localization of a white matter lesion with a vein that passes through it, termed central vein sign (CVS), is highly specific to the early stages of MS and

has been swiftly adopted as a biomarker mandated for MS diagnosis by the North American Imaging in Multiple Sclerosis Cooperative (NAIMS). The presence and development of CVS are well explained by a neuroinflammatory mechanism with a vascular origin, and CVS has been shown to differentiate well between MS and other central nervous system inflammatory vasculopathies including SLE(13) but not patients with active NP-SLE.

- *quantitative MRI (qMRI) – magnetization transfer imaging as biomarker in NP-SLE?*: Histograms of qMRI values provide a versatile and sensitive tool which is commonly applied in neurological disorders.(14, 15) They are sensitive to diffuse, global effects, and successive studies have shown the sensitivity of magnetization transfer ratio (MTR) histograms, an MTI-derived parameter, to a variety of disease related clinical and laboratory factors.(5) As several studies have highlighted, MTR is a potential marker for brain microstructural changes in NP-SLE. However, there are still several questions that must be elucidated in the near future. First, MTR histograms provide a cumulative estimation of a quantitative measure, and thus lack any spatial information. Most studies, including ours presented in Chapter 6, focused on analysis of whole brain or tissue-specific (gray matter, white matter) histograms of MTR values. Studies on SLE using this technique will need to focus on specific brain regions, either selected as a region of interest or a specific brain structure (e.g. basal ganglia). Second, MTR values are known to be low in both gray and white matter in patients with NP-SLE. The mechanism by which these values are decreased is not clear, but its reversibility upon successful treatment suggests intracellular edema and gliosis as the associated pathophysiological changes.(16) However further studies are required to fully determine whether these data reflect these changes on the brain or whether they represent the severity of NP symptoms apart from the SLE.

- *Same picture – multiple views: the role of multimodal neuroimaging in NP-SLE*: The multifactorial nature of NP-SLE, combined with the lack of specificity of most imaging modalities to any particular pathomechanism makes the quest for a "silver bullet" diagnostic tool unrealistic. A natural approach is to combine several neuroimaging markers, each highlighting a different aspect of the disease in an approach that uses a multivariate analysis in one way or the other. Several approaches for multimodal data analysis of neuroimaging data have been proposed for other neuropsychiatric and neurological disorders, especially for those with little overt brain damage and complex underlying mechanisms such as major psychiatric disorders. The application of these analyses in NP-SLE patients may help to phenotype patients and elucidate several of the underlying mechanisms.(17-19)

Improving attribution of neuropsychiatric manifestations in SLE

- *NP-SLE definition*. Since 1999, research in this field has been guided by the ACR case definitions for NP-SLE syndromes including a group of nineteen complex and uncommon

neuropsychiatric manifestations involving both the central (12 syndromes) and peripheral (7 syndromes) nervous system.(20) Researchers have mainly focused on analyzing biomarkers in NP-SLE defined as a group based in these definitions without taking into account the underlying pathophysiological mechanism. Using such heterogeneous manifestations as a group may be problematic since it may include manifestations with obviously different underlying pathophysiological mechanisms, i.e. stroke and acute confusional state. Clinicians and researchers in the field would benefit from resolving the problem of heterogeneity and the use of biomarkers capturing the different aspects of nervous involvement in SLE. Borowoy et al. demonstrated how autoantibody associations depend on the NP-SLE definition used.(21) In clinical practice, the gold standard is a diagnosis conducted by a multidisciplinary expert clinical team. Furthermore, the diagnosis in NP-SLE is made phenotypically according to the suspected underlying pathophysiological mechanism since is extremely important for guiding treatment.(22) Phenotypic characterization is important in clinical practice but may be also in research. A given phenotype may arise from a diverse set of biochemical processes and its changes in the brain may be captured by a diverse set of neuroimaging techniques. The identification of a biochemical subset of factors that underlie a certain phenotype or a certain NP-SLE manifestation should be preferable in future research.

- *Small sample size due to the low prevalence* is one of the common denominators of studies describing new potential biomarkers in NP-SLE. Given the complexity of NP-SLE, collaborative efforts, using pooled clinically, laboratory and neuroimaging data sets are needed. Much larger studies will allow for more specific hypothesis about for example a specific phenotype or NP-SLE manifestation or for example permit the use of biomarker combinations and analyze the relations among them.

- *Study design NP-SLE vs. SLE*. A reason for the minimal clinical impact of reported biomarkers may be that most of these studies report differences between NP-SLE patients and SLE at a group level while physicians have to make clinical decisions individually. Furthermore, in clinical practice, when a SLE patient presents with NP complaints obligates first to exclude other potential causes before these symptoms are attributed to SLE or to other etiologies. Most of the studies compare the higher presence of a certain biomarker in SLE patients with and without NP-SLE manifestations, remaining uncertain if this biomarker profiles are unique to NP-SLE or may be present in other mimicking neuropsychiatric disorders; only a few studies have used a group of patients with other neuropsychiatric disease (i.e. MS or septic meningitis) as control groups.(23) For example, B-cell activating factor of TNF family or matrix metalloprotease-9 have been proposed as exploratory biomarker in NP-SLE because its higher positivity when compared with SLE; however both biomarkers have been also validated as biomarkers in patients with MS.(24)

- *Omics*: in the last years, laboratory biomarker discovery has benefit from the development of omics technologies such as genomics or immune-proteomics, which has successfully increased the list of exploratory biomarkers in many diseases.(25) These techniques give the opportunity to explore a wide spectrum of biomarkers in a more comprehensive and unbiased way. Autoantigen microarrays have already been used in NP-SLE.(26, 27) For example, van der Meulen et al. have shown how a profile of IgG and IgM autoantibodies against 15 antigens may help to differentiate NP-SLE from non-NP-SLE.(26) The potential for false positive discoveries using these techniques is high; reproduction of this data and selection of best candidates may be a next step before validation in large-scale independent cohorts.(28)

- *Machine learning:* the application of the previous techniques in NP-SLE will produce hundreds of exploratory biomarkers. Analytical methods such as supervised *machine learning* (ML) promise help solving this problem and advance the development of biomarkers in the near future. This technique uses algorithms to automatically extract information from data that can be applied at the individual level to make predictions therefore with a higher level of clinical translation. This technique can be applied to laboratory biomarkers but also to neuroimaging data, since ML methods are sensitive to spatially distributed and subtle effects.(29)

Challenges and future perspectives in the treatment of NP-SLE

Drugs in Development

Advances in the understanding of immunopathogenesis of SLE have led to the development of immunotherapies targeting B cells, T cells, the costimulatory modulation, and cytokines. Although pathogenic mechanisms in NP-SLE are still poorly understood and experimental models using these new therapies are lacking, we could speculate about the potential role of some of these drugs in the future treatment of these manifestations. The promising effect of rituximab, a chimeric monoclonal antibody directed against the B-cell-specific antigen CD20, may suggest an important contribution of B cells to NP-SLE pathogenesis. Belimumab, a humanized monoclonal antibody targeted against B lymphocyte stimulator (BLyS), is now licensed in the US and Europe for the management of SLE. The BLISS trials were neither designed nor powered to definitively demonstrate the efficacy of belimumab in specific organ systems. Other trials on therapies targeting BLyS, such as tabalumab (phase II) and blisibimod (phase III) are ongoing. Atacicept, a humanized fusion protein that binds BLyS and APRIL (a proliferation-inducing ligand) has also been tested in SLE patients.(30) Both BLyS and APRIL were shown to be elevated in the CSF of SLE patients. Furthermore, they are produced locally in the astrocytes. Hence, antagonists of these cytokines could have beneficial effect in these patients; however, patients with severe CNS manifestations were excluded from all these trials, which will limit any conclusion in this respect.(31)

Several drugs targeting cytokines that are thought to contribute to the pathogenesis of both SLE and NP-SLE are currently being tested. For example, IFN-α is considered one of the most promising therapeutic targets in SLE. Sifalimumab, a human anti-IFN-α monoclonal antibody, and rontalizumab, a humanized monoclonal antibody IgG1, have shown promising results in reducing SLE disease activity across multiple clinical measures.(32) Although not confirmed in all studies, IFN-α is one of the inflammatory mediators related to NP-SLE pathogenesis. Type I IFNs are found in glia and neurons. Among their functions, IFNs induce other inflammatory mediators such as IL-6, alter brain neurotransmitters such as serotonin, and generate brain toxic metabolites. Subsequently, IFN-α has been hypothesized as a potential target in NP-SLE.(33) However, in most of trials, CNS involvement was an exclusion criterion, and the potential to treat NP-SLE will remain unknown.(31) Several studies have confirmed the intrathecal presence of higher levels of other cytokines (tumor necrosis factor (TNF)-a, IL-6, and IFN-γ) in NP-SLE. The overproduction of these cytokines is thought to play a role in the pathogenesis and severity of NP symptoms, and they have been proposed as candidate targets for future treatment.(34) Ischemic NP-SLE, especially in the presence of aPL or antiphospholipid syndrome (APS), may benefit from new-generation direct oral anticoagulants in the future, including dabigatran etexilate, a direct thrombin inhibitor, and rivaroxaban, apixaban and edoxaban, which are direct anti-Xa inhibitors.(35) Although not currently recommended in APS, these therapies may represent a potential alternative for long-term anticoagulation in APS. Rivaroxaban has shown good results in both arterial and venous thrombosis; however, information is controversial [191, 192].(36, 37) More data will be drawn from ongoing studies.

Potential Future Targets

Many modulators of the integrity of the BBB have been proposed as a potential future target to treat NP-SLE. Among them, anti-endothelial cell antibodies, complement components, cytokines and chemokines, and environmental mediators have an essential role.(38) It has been speculated that ameliorating the disruption of the BBB may have an important effect in the control of NP-SLE. Studies in MRL/lpr mice, accurately reflecting human NP-SLE, have shown the importance of TWEAK, a pro-inflammatory cytokine member of the TNF superfamily, and the alternative complement cascade in BBB disruption. TWEAK variably induces cellular proliferation, angiogenesis, apoptosis, and the production of metalloproteinase, cytokines, and chemokines.(39) TWEAK has been found to be increased in the cerebral cortices of MRL/lpr mice. Furthermore, in a murine knockout model for its receptor Fn14, mice were found to improve in cognitive function and to have less depression and anhedonia.(40) Complement component C5 has been reported to play a role in the maintenance of the BBB in mice.(41) Selective inhibition of C5aR alleviated CNS lupus.(42) Also, inhibition of the classical and alternative complement cascade with the complement inhibitor Crry was

demonstrated to alleviate experimental CNS lupus in mice.(43) Furthermore, complement plays a role in microvascular injury. Mice deficient in C3 and C5 components are resistant to enhanced thrombosis and endothelial cell activation induced by aPL antibodies, indicating the important role of alternative pathway complement activation on aPL antibody-mediated thrombogenesis.(44) Based on this information, eculizumab, a humanized monoclonal antibody blocking the generation of terminal complement components C5a and C5b-9, may be a potential drug to be used in the future in NP-SLE.(45)

Final comments

In this thesis we have analyzed an important number of laboratory, radiological, clinical and patient´s reported outcomes in SLE patients presenting with NP manifestations. Our studies are among the most robust to date in this field due to the large number of patients included, the prospective character and the standard assessment followed by a multidisciplinary expert consensus. Furthermore our studies include the novelty of a phenotypic characterization of all NP manifestations according to the suspected underlying pathophysiological mechanism (inflammation or immune-mediated vs. ischemic or thrombotic). Our studies have given more light to the understanding of the underlying pathophysiological mechanisms of nervous involvement in systemic lupus erythematosus.

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