Evaluation and optimization of laboratory criteria for Antiphospholipid Syndrome Diagnosis

Appendix II

Impact

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The main purpose of this thesis was to explore the optimization of the laboratory diagnosis of the antiphospholipid syndrome (APS). The detection of antiphospholipid antibodies (aPLs) is problematic due to the lack of standardization. Moreover, we showed the results of a multicenter study in which we investigated a newly developed aPLs-assay that might be a useful tool in the diagnosis and management of APS.

APS: a societal and economic burden

APS patients suffer from a systemic autoimmune disease characterized by recurrent venous or arterial thrombosis and/or pregnancy morbidity most likely caused by persistent aPLs [1]. APS affects approximately 1 on 2000 people[2], and is therefor one of the most common causes of acquired hypercoagulability and miscarriages in people under 50 years of age. The median age of disease onset is 31 years [3]. APLs-related vascular events exert a strong clinical impact in terms of morbidity and mortality. aPLs positivity was observed in patients with deep vein thrombosis, myocardial infarction, stroke and pregnancy morbidity [4]. Other non-criteria clinical manifestations are also frequently reported, as e.g. thrombocytopenia, autoimmune haemolytic anaemia, livedo reticularis, superficial thrombophlebitis, nephropathy, cognitive dysfunction, skin ulcers, epilepsy and cardiac valve dysfunction. Moreover, the catastrophic variant of APS (CAPS) is a serious, life-threatening aPLs-related manifestation characterized by the acute development of extensive thrombosis, leading to failure of three or more organs in less than a week. Despite treatment, mortality rate is still high and ranges between 30% to 50% [5]. Such epidemiological evidence implies that APS diagnosis and management carries an enormous social and economic costs.

Improving the diagnostic laboratory for APS

Laboratory diagnosis of APS: current problems

As the clinical symptoms of APS occur frequently irrespectively of the syndrome, classification of APS predominantly depends on a combination of different laboratory assays measuring the presence or function of aPLs. These laboratory criteria include one functional coagulation assay, known as lupus anticoagulant (LAC), and two immunological assays measuring anti-cardiolipin (antiCL) or anti-beta2-glycoprotein I (antiβ_2GPI) immunoglobulin (Ig) G and/or IgM. Positive tests should be repeated and reconfirmed at least 12 weeks apart [1]. Currently, the detection of aPLs show large inter-platform and -laboratory variation. The heterogeneity of aPLs and the lack of standardization of these assays makes the laboratory diagnosis of APS challenging.

Heterogeneity of aPLs

The antiCL assay detects different types of antibodies: antibodies directed against cardiolipin itself, directed against a complex of cardiolipin and β_2GPI and directed against other cardiolipin-binding proteins [6]. AntiCL antibodies directed against cardiolipin itself are thought to be infection-related and transient [7]. Therefore, antiβ_2GPI assays using immobilized β_2GPI have a better specificity for the laboratory diagnosis of APS. Studies have shown that aPLs that recognize the cryptic epitope G40-R43 on domain I (DI) of β_2GPI have been proven to be pathogenic, while antibodies against other domains of β_2GPI appear to be unrelated to the clinical symptoms of APS.

Detection of antibodies against thrombosis-related DI epitope

Various assays for detecting antiDI antibodies have been developed and are expected to improve the laboratory diagnosis of APS. However, no consensus is reached on whether detecting antiDI antibodies is of added value for the classification of APS (**chapter 2**). Chemiluminescence (CLIA) is currently the most widely used method to detect antiDI IgG antibodies, whose presence is a strong indicator for clinical manifestations of APS. We found that the antiDI CLIA was less sensitive but more specific compared to the laboratory criteria aPLs tests. However, it hardly improved the Odds Ratio for the occurrence of thrombosis or pregnancy morbidity. Therefore, our study demonstrated that measuring antiDI IgG by CLIA was not of added value on top of the current criteria (**Chapter 3**). A possible explanation could be the high variability in exposure of the G40-R43 epitope on DI. This epitope has proven to be cryptic and only exposed when β_2GPI is in its open conformation.

Interestingly, our in-house antiDI ELISA that ensures enough exposure of the cryptic epitope, was able to detect more samples with clinical manifestations of APS, which resulted in a higher sensitivity compared to the antiDI CLIA assay (**Chapter 4**). Taken together, our results suggest that this antiDI ELISA is able of detecting a specific population of pathogenic antiDI antibodies, thereby preventing that the patients are misdiagnosed as false negative.

Standardization of assays for detecting aPLs

Standardization of the assays used to detect aPLs antibodies in APS is of utmost importance. This standardization includes the confirmation of enough exposure of the pathogenic epitope G40-R43 on DI of β_2GPI to ascertain that at least the specific pathogenic antibody population is detected. The charge of the solid phase surface used to immobilize antigen (β_2GPI or DI) can affect the amount of exposure of this epitope. Therefore, verifying the correct exposure of the cryptic epitope in all available aPLs assays will help to improve standardization of aPLs assays and thereby patient classification.

Relevance for patients

The current APS diagnostic procedure may result in misdiagnosis of the syndrome, with major implications regarding the treatment of patients. Current treatments for APS are aimed at attenuating the procoagulant state of the patient and take into account the risk of recurrence of thrombotic events and/or pregnancy morbidities. The current treatment methods are mainly

based on oral anticoagulant therapy. Given the fact that also non-pathogenic antiβ_2GPI antibodies exist, quantitative assays measuring reactivity against the full protein, will result in false positive results. Patients with thrombosis and aPLs antibodies may be given indefinite oral anticoagulant treatment. Falsely diagnosed patients may thus be exposed to a high risk of bleeding, without having any benefit of such treatment. AntiDI assays measuring the reactivity of anitbodies against DI can improve the specificity of APS laboratory diagnosis, thereby reducing the false positive rate.

Current laboratory tests included in the criteria fail to exploit potential pathophysiological processes associated with aPLs antibodies, thereby resulting in false negative results. False-negative results also have serious consequences for patients suspected having APS because they need long-term anticoagulation to prevent recurrence. Our study firstly determined the impact of the variable exposure of the pathogenic DI epitope of β_2GPI in the commercial antiDI assays on the patient classification. Subsequently, we provided preliminary results of our in-house anti-DI hydrophobic ELISA. We demonstrated that our in-house anti-DI ELISA is able to detect the specific anti-DI antibody population against the pathogenic G40-R43 epitope, thereby improving patient diagnosis sensitivity and reducing the false negative rate. In addition, our study proved that at least this specific thrombosis-associated antibody population will not be missed when there is enough exposure of the G40-R43 epitope, thereby improving the standardization of existing aPLs assays based on the binding of aPLs to β_2GPI.

Improving risk stratification of APS patients

The current laboratory criteria include LAC, antiCL IgG and IgM and antiβ_2GPI IgG and IgM. It is sufficient for the diagnosis of APS to have one positive test when the patient is also positive for one of the clinical criteria [1]. However, not every test has the same predictive value and positivity. Therefore, risk stratification can be done by categorizing patients according to the number of positive tests and by the analysis of the aPLs profile. Each aPLs profile confers a characteristic thrombotic risk. When LAC is positive together with antiCL and antiβ_2GPI antibodies (triple positivity), it carries a significant risk for a first thrombotic event [8] and for recurrence of thrombosis [9]. Triple positivity is also an independent risk factor for pregnancy failure [10]. Our study observed that anti-DI IgG antibodies are highly correlated with triple positivity, indicating that anti-DI IgG positivity confirms the patients at higher risk for clinical events related to APS. Moreover, combined DI and triple positivity confirms an even higher risk for both thrombosis and pregnancy morbidity compared to only triple positivity (**Chapter 3**).

The LAC assay measures the functional effect of a heterogeneous group of aPLs antibodies. LAC positivity could be based on antiβ_2GPI antibodies or anti-phosphatidylserine (PS) or prothrombin (PT) antibodies, or possibly even other inhibitors. A positive LAC is considered to be a strong risk factor for thrombosis in APS. We demonstrated that an isolated LAC (in the absence of antiCL and antiβ_2GPI IgG and IgM) was also strongly correlated with a history of thrombosis with an even higher predictive value for thrombosis than triple positivity. The presence of anti-PS/PT antibodies could not explain LAC positivity in isolated LAC. As samples were negative for

antibodies against β_2GPI and PT, further studies are needed to identify other target antigen responsible for isolated LAC activity (**Chapter 7**).

Relevance for patients

Identifying the presence of factors associated with a high risk for thrombotic and/or obstetric events is critical for patient management. Our study demonstrated that a major risk factor is: the presence of LAC, the presence of triple (all three subtypes) aPLs positivity or antiDI IgG positivity. Improving the risk stratification of patients can better define high-risk and low-risk aPLs profiles and better describe the risks associated with different aPLs profiles to improve patient management.

A high-risk aPLs profile not only indicates the first clinical event[8], but also suggests recurrence [9]. Clinical decision may be modified if APS patients have a high-risk aPLs profile. For patients with APS and a first unprovoked venous thrombosis, it is recommended to receive long-term treatment with vitamin K antagonists (VKA) [11]. While for patients with a provoked first venous thrombosis, when patients have a high-risk aPLs profile, longer anticoagulation could be considered to avoid recurrence. Based on the current evidence, treatment with direct oral anticoagulants (DOACs, such as Rivaroxaban) is not recommend in triple aPLs-positive patients with obstetric APS and APS with arterial events, due to the high risk of recurrent events[12,13] . Furthermore, for women with prior obstetric APS, if an individual has a high-risk profile, it is recommended to combine treatment with low dose aspirin heparin during pregnancy.

Conclusion

The results of our study provide evidence for an update of the diagnostic criteria including risk stratification for the diagnosis and management of APS. Hereby is assay standardization of utmost importance. Subsequently, the improved diagnosis and risk stratification should improve the current treatment procedures and reduce APS-related morbidity and mortality. In addition, our findings open the way for investigating new potentially important antigen targets in the pathogenesis of APS.

References

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