

## Editorial Focus

# Revision of the Sapporo criteria for the antiphospholipid syndrome – Coming to grips with evidence and Thomas Bayes?

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The antiphospholipid syndrome (APS) has preoccupied haematologists and obstetricians since its first description as a disease entity by rheumatologists some 20 years ago (1). At that time it was discovered that many patients with systemic lupus erythematosus (SLE) who suffered from thrombotic events or recurrent abortions had either anticardiolipin antibodies (aCL) or the lupus anticoagulant, an immunoglobulin that interferes with phospholipid-dependent clotting reactions. The association of the APS and SLE turned out to be clinically very relevant. About 25–45% of SLE patients develop aCL or lupus anticoagulants (2–4). These antibodies are associated with an increased risk for thrombosis (5). Long term survival of SLE patients with a diagnosis of APS (see below) is apparently reduced (3).

It was soon observed that the APS was not confined to patients with SLE. This was defined as primary APS as opposed to secondary APS in the context of other autoimmune diseases, mainly SLE. Meanwhile, many other clinical manifestations of the APS have been described but none of them became part of the definition of the disease. Furthermore, it was observed that the specificity of antiphospholipid antibodies was not confined to cardiolipin. One of the most intriguing findings in the early 1990s was that aCL activity in most APS patients depends on the presence of a protein cofactor, usually  $\beta_2$ -glycoprotein I ( $\beta_2$ GPI) (6). In fact, many APS patients have antibodies that react with purified  $\beta_2$ GPI. Therefore, many investigators postulate that  $\beta_2$ GPI is the relevant autoantigen in APS rather than cardiolipin. Along with the detection of additional clinical manifestations and diverse autoantibodies, the definition of the APS became increasingly difficult.

Therefore, scientists have struggled to refine diagnostic criteria for the APS to improve diagnosis. In 1999 the so called Sapporo criteria for APS were presented (7), which were intended to be used in the context of clinical trials and scientific investigations, but have been used widely to diagnose the disease. These recommendations could not resolve one major problem in diagnosing APS, namely the fact that diagnosis relied on the presence of one clinical manifestation and one laboratory criterion,

which is quite different from other systemic autoimmune diseases, e.g. SLE. The dilemma posed by the definition of APS is easily demonstrated. Every patient with deep vein thrombosis fulfills already one clinical criterion. Consequently, the diagnosis of APS depends only on the presence or absence of the lupus anticoagulant or aCL. If we assume that two different tests for aCL are used, it is well possible that a thrombosis patient tests positive in one and negative in the other. How can we decide which test result is correct? Going back to the guidelines will not provide any answer to this fundamental question. In fact, there is no simple way to decide this question. Common sense suggests that the test that correlates better with future manifestations of the APS, i.e. recurrent thrombosis or abortion, is perhaps the better test. It is obvious that this question can only be resolved in appropriately designed prospective trials. The second very similar question is whether we need more sensitive or more specific tests. Again we do not know. Recent data from various clinical trials suggest that the major problem in diagnosing APS at present is not lack of sensitivity but lack of specificity. How else should we interpret the fact that in one of the largest prospective studies on the role of antiphospholipid antibodies, the Antiphospholipid Antibodies and Stroke Study (APASS) approximately 40% of stroke patients tested positive for aCL or the lupus anticoagulant, but these antibodies had no predictive power for recurrent stroke (8).

On this background Miyakis et al. propose an update of the Sapporo criteria for definite APS (9). What has been changed after seven years? Some minor changes have been made that clarify the Sapporo criteria, e.g. the definition of medium and high titres of anti-cardiolipin antibodies. The time to elapse between the initial antibody test and the confirmatory test was increased from six to 12 weeks. This will likely decrease the probability to detect temporary infection associated antibodies. Furthermore, the issue of specificity of the criteria is discussed. As a result, it is recommended that APS patients with risk factors for thrombosis in addition to antiphospholipid antibodies should be stratified separately within the APS.

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However, there are two major, conceptually relevant changes in the criteria that deserve more attention and debate: i) the inclusion of antibodies against  $\beta$ 2-glycoprotein I (anti- $\beta$ 2GPI) of the IgG or IgM isotype as a sufficient laboratory criterion for the diagnosis of definite APS, and ii) the deletion of the distinction between primary and secondary APS. Aside from these two points, the caveat to use the criteria only in clinical investigation has been omitted. It is unclear, whether the authors were aware of this. However, the reader gets no hint at any such limitation of the criteria. The provision of evidence levels clearly suggests that the recommendations are based on safe ground and thus are suitable for general diagnostic use.

What about the addition of anti- $\beta$ 2GPI to the criteria? It is not nearly as well supported by scientific evidence as suggested in the consensus statement. It is notable that two unidentified members of the committee did not support this move. The statement claims that there is level I evidence (as defined as „prospective study in a broad spectrum of the representative population or meta-analysis of randomized-controlled trials“) that anti- $\beta$ 2GPI are a risk factor for pregnancy complications and level II evidence that they are a risk factor for thrombosis which justify their inclusion into the criteria.

According to the committee, level I evidence is supported by two studies (10, 11). Faden et al. (10) analyzed 510 pregnant women of whom 20 had anti- $\beta$ 2GPI. Overall pregnancy morbidity was increased, but the difference did not reach significance. On post-hoc analysis there were two cases of preeclampsia-eclampsia (10%) in the anti- $\beta$ 2GPI positive group compared to four cases in the negative group. Even if this difference was formally significant ( $p=0.021$ ), it is based on only two events in the anti- $\beta$ 2GPI positive group, which make statistical calculations at least questionable. Furthermore, one of the two patients with eclampsia in the anti- $\beta$ 2GPI positive group did not fulfill the criteria for APS, because she was negative for anti- $\beta$ 2GPI when retested at delivery. Clearly, this study does not provide level I evidence. The second study by Lee et al. (11) analyzed the role of IgA anti- $\beta$ 2GPI rather than IgG or IgM and is therefore not even pertinent to the issue.

Level II evidence for the role of anti- $\beta$ 2GPI in thrombosis is again supported by two references (12, 13). In their excellent meta-analysis of clinical trials addressing the role of anti- $\beta$ 2GPI in thrombosis Galli et al. (12) conclude „that it is difficult to establish the value of anti- $\beta$ 2GPI and antiprothrombin antibodies as independent risk factors“. It is hard to envision how this can be interpreted as level II evidence for an independent role of anti- $\beta$ 2GPI. The other evidence is taken from a brief review of the literature on the topic (13). However, since it does not contain any information on the collection of trials included or provides any statistical analysis, it cannot seriously be considered as a meta-analysis providing high level evidence.

Next, the statement claims that anti- $\beta$ 2GPI is more specific for APS than aCL. This is backed by five references. Unfortunately, none of them represent level II evidence. For example, Detkova et al. (14) report higher specificity for anti- $\beta$ 2GPI in SLE-patients (91 vs. 75%). However, this is associated with decreased sensitivity (81 vs. 95%) underlining once more that sensitivity and specificity should not be considered separately. Obermoser et al. (15) report a 7.4 % specificity of aCL for APS. There is no

indication in their manuscript how this figure was derived. The unconventional use of evidence levels in the consensus statement continues throughout the paragraph on anti- $\beta$ 2GPI, but space limitations do not permit us to discuss every citation. It is obvious that the evidence cited does not support the recommendation to include anti- $\beta$ 2GPI as diagnostic criterion. In fact, it is likely that the addition of anti- $\beta$ 2GPI will decrease specificity of the diagnosis of APS. It will definitely increase laboratory costs if it is routinely included in the diagnostic work-up of suspected APS.

The second relevant modification dismisses the distinction between primary and secondary APS. Instead, it is recommended to document the coexistence of SLE (or other disease) with APS. This may appear a semantic issue; nevertheless, the traditional distinction has some advantages. One is even mentioned in the statement, i.e. that the classification criteria have high sensitivity and specificity in patients with SLE or lupus-like disease. Even though the authors do not differentiate between sensitivity/specificity and predictive values, they have correctly observed the consequences of Bayes' theorem on conditional probabilities. While a test may work well in a population with a high prevalence of the disease in question, it may be worthless in an unselected population (see also ref. 8). Along these lines, we would like to argue that a permanent positive lupus anticoagulant or an increased aCL IgG-titre in a SLE patient with no clinical manifestations of the APS places this patient in a high risk group for thrombotic events or (if applicable) pregnancy morbidity. The significance of such a laboratory result in a non-SLE patient is at least questionable, according to available data. Thus, the distinction of APS in the context of SLE and „primary“ APS may not be perfect. However, in terms of the interpretation of laboratory tests it should not be underestimated.

In conclusion, we feel that it is regrettable that the updated consensus statement does not contribute to the solution of the problems in diagnosing the APS. The generous assignment of high evidence levels to limited study data definitely holds the risk of severely damaging credibility in the community. The statement highlights the continuing need to improve the criteria and in particular the available laboratory tests on which we have to rely. How could this be done? It is obvious that we still do not understand why some antiphospholipid antibodies are associated with disease and apparently induce it while others do not. From the literature it appears that lupus anticoagulant activity is perhaps one discriminator between pathogenic and non-pathogenic antibodies (5). The concept that dependence of antibodies on a protein cofactor such as  $\beta$ 2GPI marks pathogenic potential, is hard to prove, because no equally simple and valid serum-based assay to distinguish between cofactor-dependent and independent antibodies exists. Furthermore, *in vitro* studies with immunoglobulin fractions or monoclonal antibodies do not unequivocally support the concept. However, they are probably the only way to get an answer to the question about the pathogenic potential of antiphospholipid antibodies.

With no gold-standard laboratory test in sight, other criteria have to be improved to make diagnosis more accurate. Why do the criteria work apparently so well in SLE patients? The answer is: Because pre-test probability for APS is relatively high in these patients. Thus, the goal must be to increase pretest probability

also in non-SLE patients. While the consensus panel has briefly mentioned some factors influencing pretest probability like age, other risk factors for thrombotic events and perhaps most important other manifestations of the APS, they have not taken the next step to integrate these factors into a scoring system. We concede that this may be difficult and even premature with the available data. However, adding more inconclusive laboratory tests to

the criteria, which have no firm evidence to support their use, will not solve the problem. Instead it would be worthwhile to design the clinical trials needed to answer the open questions. Before better evidence for the use of anti- $\beta$ 2GPI tests can be presented, they should not be included in the routine laboratory panel for suspected APS.

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