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Antiphospholipid Antibodies – Detection and Clinical Importance

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1. Introduction

Antiphospholipid syndrome (APS) is a common and very devastating disease. It is an autoimmune disease, which differs from most other systemic autoimmune illnesses by its propensity to develop thrombosis. APS is defined as the co-occurrence of antiphospholipid antibodies (aPL) with the characteristic clinical symptoms. The problem exists on the laboratory side of the diagnosis which is important to estimate the risk of further thrombosis or pregnancy complications. The diagnosis has to be made based on the presence of aPL, which are very complex entities. The main objective of this chapter is to review the methods of the aPL determinations and the clinical utility of their presence. The aPL pathogenic mechanism will be shortly discussed with an emphasis on the relation between thrombosis and inflammation.

2. The short history of the research in the field of aPL

APL with the ability to prolong *in vitro* phospholipid depending coagulation times were discovered in 1952 (Conley & Hartmann, 1952) in patients suffering from systemic lupus erythematosus (SLE). Only much later, it was discovered that *in vivo* they do not act as anticoagulants (Bowie et al. 1963) and can be found not only in SLE patients but also in apparently healthy subjects. The term "lupus anticoagulant" (LA) was used for the first time in 1972 by Feinstein and Rapaport (Feinstein & Rapaport, 1972). In 1980s, it became clear that LA belongs to a group of autoantibodies directed against negatively charged phospholipids and anticardiolipin antibodies (aCL) were determined for the first time using radioimmunoassay (Harris et al., 1983). The presence of aPL was associated with many different clinical signs and symptoms. Finally for the first time a definition of APS was established in 1987 (Harris, 1987). Then, in the 1990s, antibodies to antiphospholipid protein cofactors - beta2 glycoprotein I (a β_2 GPI) and prothrombin were discovered (Bervers et al., 1991; Galli et al., 1990; Mc Neil et al., 1990).

A set of guidelines for the detection of LA were published in 1991 and 1995 and then revised in 2009 (Exner et al., 1991; Brand et al., 1995; Pengo et al., 2009). Similar efforts were started from the beginning to standardize enzyme-immunoassays to detect aCL (Pierangeli & Harris, 2008). In 1999, first changes (e.g. exclusion of thrombocytopenia) were introduced to the

definition of APS (Wilson et al., 1999) and then the definition was modified in 2006 (Miyakis et al., 2006). Since the last modification a β_2 GPI are one of the laboratory criteria of APS.

3. LA phenomenon – The most clinically relevant aPL

LA was the first discovered aPL antibody, and until now, it has been known as the most clinically relevant. Although sensitive and quantitative ELISA-based methods were developed to detect antiphospholipid antibodies, LA detected by coagulometric tests has been shown to be more associated with thrombosis (Galli et al., 2003). From the beginning, LA together with aCL were included among laboratory criteria of the APS (Harris, 1987).

The term LA is a double misnomer because these antibodies are present mainly in patients without *lupus erythematosus* and *in vivo* react as procoagulant (Jamrozik et al., 1993). The name “anticoagulant” was given because *in vitro* in phospholipid-dependent liquid-phase assays they prolong these tests acting as anticoagulants.

The detection of LA is founded on a wide range of clot-based tests and according to the guidelines three-step procedure is required. The laboratory diagnosis should be based on prolongation of a phospholipid dependent clotting tests, lack of correction of the prolonged clotting time by addition of a small amount of normal plasma and correction by the presence of the higher concentration of phospholipids.

The detection of LA is delicate and sometimes impossible when the patient is already treated with oral anticoagulants. Pre-analytical variables (e.g. quality of sample collection, centrifugation, temperature of storage) strongly influence final results. The specimen is sodium citrate anticoagulated blood which requires immediate, quick, double centrifugation. If the test is not immediately performed, the sample needs to be frozen in a deep freeze. Moreover, one of the extremely important aspects ensuring the good test results is the process of the blood collection. The smooth blood flow prevents activation of the coagulation processes.

In the past, many different tests were used in the process of LA detection, e.g.: kaolin clotting time (KCT) (Galli et al., 1995), silica clotting time (SCT) (Dragoni et al., 2001), diluted prothrombin time (dPT) (Liestøl et al., 2002), activated partial thromboplastin time sensitive/insensitive ratio (aPTT ratio) (Ames et al., 2001), Textarin/Ecarin clotting time (Triplett et al., 1993). Recently, only two following tests have been recommended for LA detection based on practicality and global experience: diluted Russell viper venom time (dRVVT) and aPTT. The use of other tests has been discouraged mainly due to the limited experience rather than their poor performance (Pengo et al., 2009). Interestingly, according to one systematic literature review, the risk of thrombosis appeared to be independent of the laboratory tests used for LA identification (Horbach et al., 1996).

Our clinical research results show a comparable performance of the LA tests based on aPTT and dRVVT as recommended by the latest guidelines for LA detection with the other tests (Swadzba et al., 2011 a). Our study group that was used consisted of 336 subjects suffering from various autoimmune diseases. We used aPTT, dRVVT and dPT tests for LA detection together with a ratio between sensitive and insensitive aPTT reagents. All LA tests performed were associated with a previous episode of thrombosis (ORs range: 3,5 to 8,4). Diluted PT dependent LA showed stronger association with the history of thrombosis than

aPTT or dRVVT dependent LAs (OR = 6,0 vs. 5,0 and 4,3 respectively). On the other hand, LA based on the ratio between sensitive and insensitive aPTT reagents showed weaker association with APS clinical symptoms than other LA tests.

Furthermore, it has been shown that $\text{a}\beta_2\text{GPI}$ are better predictors of thrombotic complications than antibodies directed against prothrombin (Swadzba et al., 1997). For this reason, various attempts have been made to specifically detect $\beta_2\text{GPI}$ dependent LAs. Two methods of $\beta_2\text{GPI}$ dependent LA identification were described (Pengo et al., 2004; Simmelink et al., 2003). The first studies indicate that the $\beta_2\text{GPI}$ dependent LAs show superior association with thrombotic complications than LAs caused by other antibodies (De Laat et al., 2004, 2001). It was confirmed recently in our study (Swadzba et al., 2011 a). The highest odds ratio for thrombosis was found for $\text{a}\beta_2\text{GPI}$ dependent LA (OR= 8,3; specificity/sensitivity=98%/15%). Because of high specificity (98%) but low sensitivity (15%) $\text{a}\beta_2\text{GPI}$ dependent LA was suggested to be the second line assay to choose the patients with the highest risk of thrombosis.

In the recent guidelines only two tests were selected because if more tests were performed the percentage of false positive results could be too high. In our study even only two tests were used the odds ratio for both (and/or) was lower than for each one separate test. LA tested by aPTT and/or dRVVT (at least one out of two positive tests), as it was recommended by the published guidelines, was associated less strongly with the history of thrombosis (OR=4,1) than any of these tests separately (OR=5,0 and 4,3 respectively). The advantage of the latter (aPTT and/or dRVVT) lies only in their higher sensitivity (45% vs. 43% for aPTT dependent LA and 32% for dRVVT dependent LA). When both tests were positive ("double LA positivity") the association with thrombosis was stronger (OR=6,5) than when only one test was positive. Double LA positivity detected by all tests performed was firmly associated with the history of thrombosis.

We agree with the recommendation, that two tests should be performed and the number of positive tests should be given and commented on the final result. The results with two positive LA tests could be named as "double LA positive" and possibly point to the patients with the highest risk of thrombosis.

LA detection is troublesome, poorly standardized, and its laboratory accuracy and clinical utility varies, igniting a lot of controversies. As a consequence several national and international inter-laboratory surveys have shown unacceptable differences in LA test results between various participating laboratories (Arnout et al., 1999; Pengo et al., 2007b). The rates of false positive and false negative results remained relatively high. In the future the differences among laboratories should be less pronounced, because the new guidelines gave more strict recommendations about blood collection requirements, choice of the tests, mixing studies (1:1 proportion), confirmatory tests, interpretation, expressing and reporting the results.

However there is still a need to conduct more research to answer a lot of questions, e.g.: how identify the most clinically relevant LA, how to define the presence of strong LA as opposed to the weak ones. From a clinical point of view, any LA results should always be considered in the context of a full laboratory aPL profile comprising of aCL and $\text{a}\beta_2\text{GPI}$, because isolated LA positivity may be frequently found in subjects without any clinical symptoms (Pengo et al., 2007a).

4. ACL – The most sensitive aPL test

ACL were firstly detected by radioimmunoassay but just after ELISA based format replaced the former tests (Loizou et al., 1985). It is well known that basic performance of aCL assays is determined by various factors including the type of microtiterplate, the source of cardiolipin, the solvents, the usage of cofactor (De Groot et al., 2008). There were a lot of efforts to standardize the aCL tests (Wong et al., 2005, Wong & Favaloro, 2008). However it is not easy because the aCL ELISA does not detect aCL, but antibodies to proteins which bind to cardiolipin coated on the surface of microtiterplate. β_2 GPI is present in the microtiterplate wells from the patient serum (tested), bovine serum (solvent) or sometimes β_2 GPI is extra added to aCL tests. β_2 GPI can bind to cardiolipin coated on the plate and later $a\beta_2$ GPI bind to the complex β_2 GPI-cardiolipin. However the cardiolipin on microtiterplate can be bound also to prothrombin and other proteins. For this reason an aCL test is not purely $a\beta_2$ GPI test, but, in fact, there are detected antibodies against different proteins which bind to anionic phospholipids. This is probably the reason why ELISAs to detect anticardiolipin antibodies are more sensitive but less specific tests than $a\beta_2$ GPI ELISAs.

Proper and stable calibrators are very important for conducting aCL tests. Almost since the beginning one source of human polyclonal calibrators from patients samples was applied (Louisville calibrators) (Harris et al., 1987). These widely used calibrators helped produce comparable results between laboratories and introduce international units (GPL and MPL units) (Harris, 1990). The Louisville calibrators were used for many years in spite of the problems with batch to batch variations (Favaloro & Wong, 2011). Some years ago monoclonal antibodies to β_2 GPI became available and they started to be used as the calibrators both for β_2 GPI ELISA and aCL ELISA (Ichikawa et al., 1999). Even though a great progress has been made, there is no consensus for internationally accepted aCL calibrators.

The other important issue in aCL testing is associated with choosing an optimal cut-off point. In the first studies, the 95th percentile of healthy population was used (Musial et al., 1997), but from the beginning it was well known that low positive results were less important than high positives. In the Sapporo APS criteria, only medium or high titer of aCL were considered as serological criterion of APS (Wilson et al., 1999). In the newest, updated criteria, aCL are regarded as positive if exceed the 99th percentile or 40 GPL/MPL units (Miyakis et al., 2006). There is an inconsistency in offering two different, alternative values to identify the presence of aCL in a meaningful, pathological levels (Swadzba et al., 2007). In our in-house method the value of the 99th percentile of a normal population corresponded to 26 GPL and 27 MPL, respectively. Obviously, the sensitivity was higher for a lower cut-off value (99th percentile), specificity – for the higher one (above 40 GPL/MPL), but overall, relative risk for thrombosis was quite similar (3,71 vs. 3,72) (Swadzba et al., 2007).

However, until generally accepted international standard is developed, any arbitrarily chosen cut-off value will leave doubts about its validity. It seems reasonable to have only one threshold value when comparisons between different methods and laboratories are required. In our opinion, the 99th percentile of the healthy population offers by far the better threshold value that could be generally accepted (Swadzba & Musial, 2009).

Nevertheless, one needs to remember that lower levels of both aCL and $a\beta_2$ GPI can be associated with significant (but low) risk of clinical complications (Swadzba et al., 2007). Even if it is persistent positivity, one low positive result could be neglected, but when two or

more aPL are present in low titer the situation can be different. We can speculate that in the recently developed definition of the “triple positivity” maybe even low positive antibodies could be enough to register as positive (when at least one kind of antibodies is highly positive).

On the contrary, patients with very high levels of antibodies, especially of the IgG class, are at particularly high risk of thrombosis. This has been clearly reflected in our study by a group of patients with anticardiolipin antibodies level above 80 GPL/MPL who had the highest number of thrombosis in the past (Swadzba et al., 2007). In our opinion, this finding requires and calls for an objective, clinically important and uniformly accepted definition of “high levels” of aPL.

It should be emphasized that the class of anticardiolipin antibodies is crucial for determining the risk of APS clinical symptoms. It was shown in many studies that IgG class of antibodies correlates much better with the history of thrombosis than IgM type (Swadzba et al., 2007). It is of concern that the updated criteria do not separate IgG and IgM classes. We propose to group separately IgG and IgM antibodies and introduce the thrombosis risk score in which IgG antibodies will be more important than IgM. Some other authors even think that IgM antibodies should be abandoned from the criteria of APS (Galli et al., 2008).

The value of IgA antibodies in diagnosis of APS is still uncertain. According to the latest guidelines this class of antibodies does not add clinically important information (Miyakis et al., 2006).

5. Anti β_2 GPI – The most “real” aPL test?

β_2 GPI is a 50-kDA phospholipid binding protein present in plasma in concentrations of approximately 200 mcg/ml. The function of β_2 GPI is not clear until now however it was regarded as natural anticoagulant. The genetic deficiency of β_2 GPI appears to be not involved with any diseases (Matsuura et al., 2010). It has been found recently that β_2 GPI can play an important role in the immunity (De Groot & Meijers, 2011). There is no evidence that any of β_2 GPI polymorphism is connected with the presence a β_2 GPI antibodies or APS (Swadzba et al., 2006).

Antibodies against β_2 GPI were discovered in 1990 and it is well documented nowadays that β_2 GPI is the main important antigen in APS pathophysiology. It was demonstrated that the mice injected with β_2 GPI produce aCL as well as a β_2 GPI (Gharavi et al. 1992). From the pathophysiological point of view, inclusion of β_2 GPI antibodies to existing criteria seems to be well-founded. It is generally accepted that domain I of β_2 GPI serves as a major antigen for pathological aPL (Ioannou & Rahman, 2010). A β_2 GPI IgG antibodies are strongly associated with clinical complications of APS. This further justifies inclusion a β_2 GPI into the APS laboratory classification criteria.

It is noted, that the presence of a β_2 GPI was closely associated with the presence of other aPL. For this reason, addition of a β_2 GPI to the diagnostic armamentarium changed only slightly the risk prediction of clinical complications based on the presence of LA and aCL. In our study their inclusion added 5 extra patients diagnosed with APS, who otherwise would be missed (less than 5% of the APS population) (Swadzba et al., 2007). Overall, the addition

of a β_2 GPI to the classification criteria might slightly limit the number of the so-called “seronegative APS” patients; a relatively small group of subjects with very suggestive clinical features and negative LA and/or aCL determinations.

A β_2 GPI, especially of the IgG class, appeared in our patients less frequently than aCL (Swadzba et al., 2007). For this reason, being more specific, they showed quite low sensitivity, as it was already reported by others (Favaloro & Wong, 2011). Overall odds ratios for thrombosis and pregnancy loss is similar to the aCL ones.

The proposed single method of establishing an upper limit of normal values for a β_2 GPI introduced by the updated APS criteria would certainly help to compare results between laboratories. The choice of 99th percentile as a suggested cut-off value seems valid and very well-supported. For example in our research studies, patients tested positive for a β_2 GPI based on a cut-off set at 99th percentile were at a higher relative risk for thrombosis than those considered positive when the cut-off value was set at 95th percentile. However, quite low sensitivity of the test (in our study for a β_2 GPI IgG – 24%) seems to qualify it rather as a risk stratification test or a second test identifying population at the highest risk among APS patients (Swadzba et al., 2007). As a screening test or as a test served in a “triple positivity” the cut-off value that lies in the 95th percentile may be better and it needs further studies.

6. Non classical aPL

The spectrum of autoantibodies associated with APS is likely to extend beyond tests known from APS criteria. From the very beginning it was known that cardiolipin is not the only one but only one out of the many possible phospholipids served in the tests for detecting aPL. It was unlikely that cardiolipin could serve as real antigen for aPL and even that *in vivo* it is a surface for the proteins because cardiolipin is situated only in the inner side of mitochondria membranes.

Other phospholipids were proposed as the antigens. From a pathophysiological point of view the phosphatidylserine was very attractive as phospholipid that exists in the cellular membranes and flip-flops to the outer side during the cell activation. In fact in our studies, the correlation with APS clinical symptoms did not differ between phosphatidylserine antibodies and aCL. The correlation between two methods was very high ($r=0,8$) (Musial et al., 2003). Antibodies against other phospholipids were tested less extensively (e.g. antibodies against phosphatidylinositol) but similar results were obtained. Due to high cross reactivity there was only a little additional clinical information leading to the conclusion that there is no need any additional ELISA tests against anionic phospholipids other than aCL. The special attention was drawn to antibodies against phosphatidylethanolamine (aPE). Phosphatidylethanolamine is a zwitterionic phospholipid normally present in the outer leaflet of cell membranes, and it plays a role in reactions of protein C pathway. Antibodies can react with the complexes of aPE with high and low molecular weight kininogens (HMWK, LMWK), factor XI and prekallikrein (Sugi & McIntyre, 2001). There were some studies conducted that show aPE as the only antibody in a limited number of APS patients (Karmochkine et al., 1992).

The method using the mixture of different phospholipids was proposed as an alternative to cardiolipin tests and it was called “the real antiphospholipid test”. The concept of the test was similar to LA (detecting antibodies against different antigens) and the aPL ELISA from

Louisville in some studies showed better sensitivity and specificity than aCL (Pierangeli & Harris, 2008). However, most of the studies used cardiolipin as the antigen that was easier to standardize and compare between laboratories than tests with different mixtures of different antiphospholipids.

As mentioned early, in 1991 antibodies to the prothrombin (aPT) were discovered (Bever et al., 1991). In the clinical studies aPT were found to be less clinically important than other aPL antibodies (Swadzba et al., 1997). However the group of researchers developed a new test recognizing clinically relevant aPT. They coated prothrombin on phosphatidylserine (PS/PT test) causing new structure configuration of prothrombin. The complex of prothrombin and phosphatidylserine is probably showing epitopes which bind clinically relevant antibodies. These antibodies rather than antibodies against prothrombin alone are closely associated with APS and LA (Atsumi et al., 2000).

Test for the presence of anti annexin V antibodies is one of the new tests especially used in obstetrical APS. Annexin V plays a significant role as an anticoagulant shield on the surface of the trophoblast. In some studies, the presence of anti annexin V antibodies correlates positively with a history of pregnancy losses (Iaccarino et al., 2011).

Anti β_2 GPI - domain I antibodies were tested recently. In contrast to antibodies recognizing other domains of β_2 GPI, anti-domain I antibodies are found to be highly associated with APS clinical symptoms, however their sensitivity is very low (De Laat & de Groot, 2011).

7. New trends in aPL determination

ELISA method is state-of-the-art for aCL and a β_2 GPI determination. It is a manual or semi-manual technology and inter-assay reproducibility is difficult to obtain. Manufacturers put a lot of effort to develop new methods. There are some new automated assays for diagnosis of the antiphospholipid syndrome on the market (De Moerloose et al., 2010; Persijn et al., 2011). These assays are two-step immunoassays consisting of paramagnetic particles coated with cardiolipin and/or human β_2 GPI. Sensitivity, specificity, agreement and the odds ratios when predicting a thrombotic or obstetric event gave comparable results with the ELISA methods.

Western blot is the other technique used recently in aPL determination (Egerer et al., 2011). It was published that it gives similar results as ELISA, although the results are only qualitative.

High avidity a β_2 GPI was associated with thrombosis and APS, while in the low avidity a β_2 GPI group non-APS (predominantly SLE) patients prevailed (Cucnik et al., 2011). Controversies concerning the role of antibody avidity may be attributed mainly to the absence of suitable detection methods.

We proposed to use ROC (Receiver Operating Curve) methodology to establish retrospectively optimal cut-off point for each clinical symptom, each type of the antiphospholipid antibodies and to determine the test with the best clinical accuracy (Musial et al., 2003). The comparison between tests based on ROC plot analysis is independent of any particular threshold values. The calculation of the area under the ROC curve (AUC) gives the information about the clinical accuracy. The area of 1,0 means that the test perfectly separates subjects with a given APS clinical symptom from those without it (an

ideal situation – 100% sensitivity and 100% specificity). When the area is $< 0,5$ the test does not separate such two groups of patients at all. ROC plot analysis is further used to find threshold values for aPL which discriminate the best between the patients who experienced an APS clinical symptom in the past from those who did not. The optimum cut-off point is the nearest point to the left upper corner on the graph of ROC curve. Such calculated cut-off value provides the optimum trade-off between the specificity and sensitivity of the test towards a particular clinical symptom.

8. APS definition and aPL clinical utility

Classification criteria for the antiphospholipid syndrome were proposed for the first time by Harris in 1987 (Harris, 1987). In the definition, he emphasized the co-existence of typical clinical complications and laboratory test abnormalities. Among laboratory tests, he listed the presence of LA and aCL, which were discovered four years earlier (Harris et al., 1983). Typical clinical manifestations included venous and arterial thrombosis, thrombocytopenia and obstetrical complications. These criteria were slightly modified during the Conference in Sapporo in 1999 (Wilson et al., 1999). Thrombocytopenia was removed as being not specific, obstetrical criteria were revised and their very detailed description was given. At the beginning of the 1990's, β_2 GPI was shown to be a major antigen for aPL. For this reason, although aCL and $\alpha\beta_2$ GPI often coexist, $\alpha\beta_2$ GPI were added to the criteria (Miyakis et al., 2006). Subsequently, it was shown that $\alpha\beta_2$ GPI were more specific, but less sensitive than aCL as markers of clinical complications of APS. Based on the updated criteria, it is recommended to divide patients into classes according to the type and number of antibodies present. Patients should be classified into class I when they possess more than one type of antibody. In our study, this group did not differ in terms of thrombosis risk from patients with the presence of only one type of aPL (Swadzba et al., 2007). Only groups with all three types of antibodies (LA + aCL + $\alpha\beta_2$ GPI) were connected with the higher risk. Class II comprises patients with the presence of only one type of antibody (IIa-LA, IIb-aCL and IIc- $\alpha\beta_2$ GPI). For thrombosis in general, and especially for venous thrombosis, the presence of LA (class IIa) brings the higher risk than groups IIb and IIc (Swadzba et al., 2007). APS patients are not routinely connected with the groups because it did not clearly differ by the risk of thrombosis or pregnancy loss.

It was shown that not only the presence of antibodies is important but also the coexistence of different antibodies can increase the risk of thrombosis. The term "triple positivity" for the presence of all classic aPL was given (Pengo et al., 2010). It is connected with high risk of thrombosis. The other high risk factors of APS clinical symptoms have been recently discovered: anti domain 1 antibodies, β_2 GPI dependent LA and double positive LA.

It is concerning, that the updated criteria do not separate IgG from IgM class of antibodies. The group of patients with combined: LA and aCL IgG or LA and $\alpha\beta_2$ GPI IgG, can have similar risk of thrombosis as the patients with triple positivity: LA and two IgM antibodies (aCL and $\alpha\beta_2$ GPI). We agree that there is little evidence demonstrating significant association between IgM $\alpha\beta_2$ GPI and aCL but still there are some patients with only high IgM positive antibodies and clinical manifestations of APS. It could be too preliminary to exclude IgM antibodies completely, so we rather suggest to make a clear distinction between antibodies of the IgG and IgM class as the high and low risk antibodies until prospective studies remove any doubts that antibodies of the IgM class, even in very high titers bring negligible risk of thrombosis.

Undoubtedly, laboratory criteria that define the antiphospholipid syndrome require modification because the two different possible cut-off values for discrimination between positive and negative aCL (>99th percentile and >40 GPL). In 90 aCL-positive APS patients it was shown that the values defining 99th percentile of the normal population (17,4 GPL and 26,8 MPL) were significantly lower than 40 GPL (Swadzba et al., 2007); this finding was already reported by our group about some years earlier (26 GPL and 27 MPL, respectively) (Musial et al., 2003). We suggest to change the criteria and start using the 99th percentile as the only accepted cut-off value (as for anti- β_2 GPI antibodies). Interestingly, Ruffatti (Ruffatti et al., 2008) proposed: one criterion to be used for thrombosis (40 GPL) and the other (99th percentile) for pregnancy complications. In group of their patients the mean value of aCL IgG was higher in thrombosis than in the group with obstetrical complications. It was concluded that lower titers can provoke obstetrical complications and are not enough for stimulating thrombosis. In our group of patients, the results were quite different. Using ROC curves the best cut-off for thrombosis was 17,2 and for recurrent fetal loss slightly higher 18,4 (Wu et al., 2008). A certain percentile of healthy population is the objective method for determination cut-offs regardless of the test used. It is important because there are many debates related to what percentile (i.e., 95th, 97,5th or 99th) is the best to use. This is the problem of optimal sensitivity/specificity ratio. The ROC curve methodology can show the best cut-off to reach optimum ratio between sensitivity and specificity and the highest OR for clinical symptoms connected with positive values of the tests. In 2003 we published cut-offs based on ROC curves for different antiphospholipid antibodies. Optimal cut-off for aCL IgG was slightly lower than 99th percentile (26 GPL) and far lower than 40 GPL. We regard it as correct that 99th percentile should be included in the criteria of APS but 95th percentile can be used as for a group with doubtful antibodies or in triple positivity when two others antibodies are positive.

Another radical proposition is to abandon aCL determinations completely, which is bringing up the argument of high specificity of a β_2 GPI for clinical APS symptoms. However we should not forget about sensitivity, which is quite low for these antibodies. Also, it has not been proven beyond any doubts that aCL antibodies directed against other proteins than β_2 GPI are with no clinical importance. ACL like LA measure a cocktail of antibodies against different proteins and epitopes. From a practical point of view, aCL tests are much cheaper, better known by the clinicians and give similarly important clinical information.

APL positive patients with no APS clinical symptoms are at present excluded from the diagnosis of APS syndrome. We agree that the term “syndrome” is connected with any clinical symptom. If we agree that “triple positivity” gives high risk for thrombosis even if there were no clinical APS symptoms we should consider to develop a new definition. The proposition could be aPL dependent thrombophilia. LA and persistently high titers of all groups of aPL antibodies in IgG class probably are connected with even higher risk of thrombosis than moderate aCL of IgM class in women with one pregnancy loss in the history.

9. APL – A pathogenic mechanism, the role of inflammation in thrombosis

The coexistence of aPL and clinical signs of APS is obvious but causative role of aPL is not certain. There are some theories how aPL can cause thrombosis or abortions. Most of them are connected with prothrombotic and antifibrinolytic mechanisms. Relations between thrombosis and inflammation are researched recently.

Tumor necrosis factor alpha (TNF- α) is a cytokine which shares proinflammatory and prothrombotic actions, while a soluble form of interleukin-2 receptor (sIL-2R) is considered as a typical marker of (auto)immune inflammation with not known direct links to thrombosis. The differences in the pathogenesis of APS as compared to other autoimmune diseases might be connected with different serum levels of both mediators (Swadzba et al., 2011 b).

APS was characterized in our study by the highest levels of TNF- α . Moreover, patients with *lupus anticoagulant* or elevated levels of IgG anticardiolipin or IgG anti- β_2 -glycoprotein I antibodies had higher TNF- α levels than patients without the presence of any type of antiphospholipid antibodies. The presence of aPL is associated with higher TNF- α level, whereas increased level of sIL-2R is rather connected with definite SLE where inflammatory processes prevail. It might be hypothesized that TNF- α plays a major role in pathogenesis of APS thrombotic phenomena.

In general, systemic inflammation is a potent prothrombotic stimulus. Inflammatory mechanisms upregulate procoagulant factors, downregulate natural anticoagulants and inhibit fibrinolytic activity (Esmon, 2003). Endotoxin, tumor necrosis factor alpha and interleukin-1 α (IL-1 α) induce tissue factor (TF) expression, primarily on endothelial cells and monocytes/macrophages, promoting blood coagulation (Bevilaqua et al., 1986). Activation of the complement C5b-C9 complex changes the cell surface to a more procoagulant phenotype by the shift of negatively charged phospholipids from the inner to the outer membrane (Sims et al., 1988). Inflammatory reaction is also accompanied by the increase in fibrinogen and C-reactive protein (CRP) blood levels. CRP itself increases TF and decreases TF pathway inhibitor (TFPI) concentrations, what may be important in pathogenesis of arterial thrombosis and myocardial infarction (Wu et al., 2008). Of the natural anticoagulants, protein C pathway appears to be the most strongly influenced by inflammation with thrombomodulin (TM) and the endothelial cell protein C receptor (EPCR) being both downregulated by TNF- α (Conway & Rosenberg, 1988).

On the other hand, thrombotic processes enhance inflammatory reactions, mainly through the action of TF and thrombin (Chu, 2005). Activation of platelets leads to the release of CD40 ligand, which in turn induces TF expression and increases interleukin 6 (IL-6) levels (Miller et al., 1993). Thrombin also augments leukocyte adhesion and activation, stimulates endothelial cells to produce platelet activating factor (PAF) and increases an expression of P-selectin (Pierangeli et al., 2001).

A common inhibitory pathway for thrombosis and inflammation also exists. Activated protein C (APC) acting directly as an anticoagulant, functions also as an anti-inflammatory and cytoprotective agent through specific receptors: EPCR and protease activated receptor-1 (PAR-1) (Crawley & Efthymiou, 2008).

It is possible that TNF- α is a proinflammatory cytokine with the strongest prothrombotic action. TNF- α stimulates monocyte and neutrophil adhesion to endothelium, inhibits protein C system, impairs fibrinolysis and increases TF expression on the cell surface (Esmon, 2003). Produced mainly by activated monocytes, macrophages, and T lymphocytes, this cytokine has been found to be elevated in patients suffering from both SLE (Studnicka-Benke et al., 1996) and APS (Forastiero et al., 2005). We hypothesized that TNF- α might be more elevated in APS patients, with thrombosis as its prominent feature, than in those with other autoimmune diseases where immune mediated inflammation prevails.

10. Conclusion

In summary, an updated list of APS classification criteria and a revision of LA guidelines represent a step forward for better detection of clinically important aPL. However, many problems still need to be addressed and an additional research in this field followed by new modifications in definitions and criteria seems to be necessary. The important issue is to find optimal methods for high risk aPL detection. This may lead to the development of an effective primary and secondary prophylaxis of clinical APS complications, which constitutes a major goal and challenge for the future.

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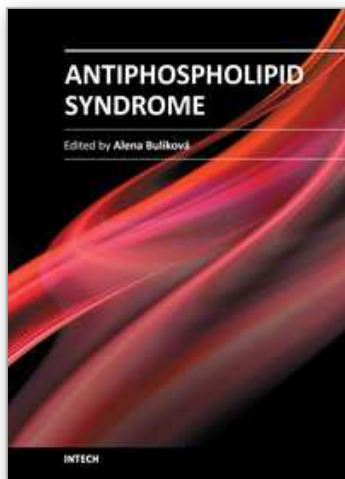
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The antiphospholipid syndrome has been described for the first time by Graham Hughes in 1983 as a condition connected with thromboses or foetal losses and antiphospholipid antibodies presence. From that time there has been a great progress in knowledge, including antiphospholipid antibodies characterisation, their probable and also possible action, clinical manifestations, laboratory detection and treatment possibilities. This book provides a wide spectrum of clinical manifestations through Chapters written by well known researchers and clinicians with a great practical experience in management of diagnostics or treatment of antiphospholipid antibodies' presence.

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