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SPECIAL ARTICLE

'Non-criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, TX, USA, April 2010

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Abstract: Current classification criteria for definite APS recommend the use of one or more of three positive standardized laboratory assays, including anticardiolipin antibodies (aCL), lupus anticoagulant (LA), and antibodies directed to $\beta_2$glycoprotein I (anti-β_2GPI) to detect antiphospholipid antibodies (aPL) in the presence of at least one of the two major clinical manifestations (i.e., thrombosis or pregnancy morbidity) of the syndrome. Several other autoantibodies shown to be directed to phospholipids and/or their complexes with phospholipids and/or to proteins of the coagulation cascade, as well as a mechanistic test for resistance to annexin A5 anticoagulant activity, have been proposed to be relevant to APS. A task force of worldwide scientists in the field discussed and analyzed critical questions related to 'non-criteria' aPL tests in an evidence-based manner during the 13th International Congress on Antiphospholipid Antibodies (APLA 2010, 13–16 April 2010, Galveston, Texas, USA). This report summarizes the findings, conclusions, and recommendations of this task force. *Lupus* (2011) **20,** 191–205.

Key words: autoantibodies; prothrombin; phosphatidylethanolamine; IgA

Introduction

Current classification criteria for definite antiphospholipid syndrome (APS) recommend the use of one or more of three positive standardized laboratory assays to detect antiphospholipid antibodies (aPL) in the presence of at least one of the two major clinical manifestations (i.e., thrombosis or pregnancy morbidity) of the syndrome.[1] Anticardiolipin antibodies (aCL), anti-$\beta_2$glycoprotein I (β_2GPI) antibodies, and lupus anticoagulant (LA) are the laboratory tests included in the revised criteria for the classification of APS.

A number of issues regarding the definition of 'aPL positive' are under discussion. For example, there are in daily practice many in vitro 'false positives' for aPL, due to the lack of specificity of the tests, particularly the aCL ELISA. APL antibodies are found in patients with a variety of diseases, such as infectious, malignant, or autoimmune diseases (clinical false positive), but in those cases they are

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not associated with clinical manifestations of APS. Furthermore, increasing evidence demonstrates that aPL antibodies are heterogeneous in function and specificity, and individual tests may recognize various subtypes of antibodies, some of which may be 'pathogenic'. In addition, there are patients strongly suspected of having APS by their clinical phenotype, but persistently negative for any currently tested aPL (laboratory and/or clinical false negative). These findings have nurtured the concept of 'seronegative APS' (SNAPS), a much contended setting that is based on a clinical picture highly suggestive of the syndrome in the absence of conventional aPL antibodies, leading investigators to maintain their efforts to identify 'true aPL' in an attempt to better recognize APS patients.

Several autoantibodies shown to be directed to negatively charged phospholipids other than cardiolipin, to other proteins of the coagulation cascade (i.e., prothrombin and/or phosphatidylserine–prothrombin complexes), to some domains of β_2GPI, or to interfere with the anticoagulant activity of annexin A5 (A5), have been proposed to be relevant to APS.[2] In some cases, these assays appear to detect specific subsets of pathogenic antibodies, or a particular mechanism in APS. However, the clinical utility of these newly developed assays and their diagnostic value remains elusive. The issue of the value of IgA aPL antibodies and whether this test should be part of the routine diagnostic algorithm has also been a subject of debate. A worldwide task force of scientists in the field – divided into subgroups – discussed and analyzed critical questions related to 'non-criteria' aPL tests in an evidence-based manner during the 13th International Congress on Antiphospholipid Antibodies (APLA 2010, 13–16 April 2010, Galveston, TX, USA). This report summarizes the findings, conclusions, and recommendations of this task force.

Antibodies to phosphatidylethanolamine

(Presented by Drs Sanmarco, Lambert, and Matsubayashi)

Introduction and questions addressed by the task force

Antibodies directed toward phosphatidylethanolamine (anti-PE) deserve particular attention, since they have been described in some instances as the sole aPL in patients that have manifestations of APS. Thus, the goal of this session was to highlight the clinical interest of anti-PE investigation through a brief review of the literature of their clinical associations and clinical experience. Another point opened to the debate was the methodological problems of the anti-PE assays.

Regarding obstetrical complications, anti-PE have been reported to be significantly more frequent in women with unexplained early fetal loss (UFL) than in either those with explained early fetal loss or healthy mothers. Two different studies have shown that anti-PE are a higher independent risk factor for early UFL than either aCL or anti-β_2GPI antibodies.[3,4] Interestingly, anti-PE have also been described as the only aPL found in the majority of cases (73%). Likewise, anti-PE have been reported as significantly the most frequent aPL in infertile women (67.5% of aPL-positive sera), where they were found to be the sole aPL in 85% of cases.

Recently, a murine model has reinforced the interest in anti-PE investigation in obstetric complications. Indeed, as reported by Dr Matsubayashi in this session, passive immunization of anti-PE or anti-LDC27 (antigen site in the third domain of kininogen) in pregnant mice causes increased fetal resorption, which correlated with significant increases in apoptosis in the placenta (study in progress). He claimed that this study supports the pathogenic role of anti-PE in pregnancy complications and also suggests the importance of LDC27, the target antigen site for kininogen-dependent anti-PE.

The relationship between anti-PE and thrombosis, the other clinical feature of APS, has also been reported in several studies. In particular, in a multicenter study set up within the framework of the European Forum on aPL, the prevalence of anti-PE was 15% in patients with unexplained venous thromboses and mainly found as the sole aPL.[5] In this retrospective study, IgG-anti-PE were found to be an independent risk factor for venous thrombosis, with an odds ratio of 6:1. Interestingly, Dr Lambert reported that in a selected population of 243 outpatients consulting for idiopathic arterial and/or venous thrombosis, negative for conventional aPL antibodies, 58 were positive for anti-PE (IgM mainly and IgG rarely). Other thrombophilic disorders were not frequently found. During a median follow-up of 34 months, thrombotic recurrence was found in 25% of patients.

Importantly, the task force recognized that no consensual standardized method exists for the measurement of anti-PE and that the heterogeneity of these antibodies increases the difficulties in attempting such a goal. This problem significantly limits

the clinical utility of this assay. The impact of the various ELISA components on the interlaboratory variability of results was analyzed, the conclusion being that the buffer supplement represents the critical factor in anti-PE measurement. To that regard, the results from a recent study showing that buffer supplements with a high lipid content decrease anti-PE reactivity in a dose-dependent manner were presented at this meeting.[6]

Recommendations of the task force

Based on published evidence and the additional studies presented during this session, the detection of anti-PE antibodies may be useful in 'seronegative' APS, in spite of the absence of a consensual method for their detection. The task force recognized that further steps must be made in order to ascertain the place of these antibodies in the diagnostic algorithm of APS, including standardization and proper validation of an anti-PE ELISA test and a prospective study on a broad population with well-documented clinical and biological features of APS (Table 1a).

Antibodies to domains of β2 glycoprotein I

(Presented by Dr Bas de Laat)

Introduction and questions addressed by the task force

APL antibodies form a heterogeneous population of antibodies recognizing different antigens.[7] β_2GPI is recognized as the most important antigen in APS, but anti-β2GPI antibodies are also regarded as a heterogeneous population of antibodies with reactivity towards different epitopes on β_2GPI.[8] During the last decade evidence has accumulated for a central role for domain I of β_2GPI as a primary epitope for aPL antibodies. Iverson et al. were the first to show that a specific population of aPL antibodies showed reactivity towards domain I, with glycine40-arginine43 as the major epitope.[9,10] Recently Ioannou et al. reported that the epitope possibly comprises a larger region on domains I and II.[11]

Two studies have been conducted to investigate the clinical significance of the detection of anti-domain I antibodies. The first of these showed that the presence of anti-domain I antibodies was associated more with (predominantly venous) thrombosis compared with anti- β_2GPI antibodies with reactivity towards other domains.[12] This observation was recently confirmed in a double-blinded multicenter study including 442 patients, all positive for anti- β_2GPI antibodies.[13] Anti-domain I antibodies were shown to be present in the plasma of 243/442 patients (55%). From these patients with anti-domain I antibodies in their plasma, 83% had a history of thrombosis resulting in an odds ratio of 3.5:1 (2.3–5.4, 95% confidence interval, CI) for thrombosis. Interestingly, it was also found that anti-domain I antibodies were associated with pregnancy morbidity. Furthermore, recently in vivo data have been generated with respect to domain I. Ioannou et al. conducted a study in which mice were injected with IgG purified from patients diagnosed with APS.[14] After standardized vessel injury, mice injected with antiphospholipid-related IgG displayed increased thrombus size that could be inhibited by domain I of β_2GPI.

This task force subgroup was charged with investigating whether there is sufficient scientific evidence to recommend the incorporation of the assay to measure anti-domain I antibodies for implementation in the official guidelines for diagnosis of patients with APS.

Recommendations of the task force

The general opinion of the task force was that detection of anti-domain I antibodies is of major importance. This was predominantly based on a double-blinded multicenter study in which it was shown that anti-domain I antibodies were associated more with thrombosis and pregnancy morbidity compared with antibodies with reactivity towards other domains of β_2GPI.[13] One of the problems that can also be applied to (some of) the other assays that are already included in the official guidelines is lack of prospective data (a) and causality (b):

(a) Several prospective studies have been performed with regard to the clinical significance of the presence of aPL antibodies regardless of specificity, but there is no consensus as to whether the presence of aPL antibodies is a risk factor for thrombosis (either first or second event).[15–21]
(b) The causality of anti-domain I has been demonstrated only by the use of animal models, and additional clinical studies are needed.[14]

Therefore, this task force recommended that the anti-domain I assay may be used in a research-based setting and that more prospective and in vivo data are needed before the anti-domain I

Table 1 Questions and recommendations of the non-criteria aPL task force

1a. Anti-PE antibodies and antibodies to negatively charged phospholipids other than cardiolipin

Test	Questions addressed by task force	Recommendations
Anti-PE antibodies	Is the anti-PE ELISA standardized? What are the challenges with the assay?	Standardization of anti-PE ELISA needed
	Are anti-PE antibodies clinically relevant?	Well-designed clinical studies needed to confirm the diagnostic value of anti-PE antibodies
Antibodies to negatively charged phospholipids other than cardiolipin		
a) Perspectives and experiences from a large reference laboratory in the USA	Are antibodies to negatively charged phospholipids other than cardiolipin important in the diagnosis of APS?	Important to establish whether these antibodies recognize additional APS patients, currently missed with traditional assays Address existing technical problems and inconsistencies with the tests Anti-PS may be best candidate with respect to relevance and association with recurrent pregnancy loss
Antibodies to negatively charged phospholipids other than cardiolipin		
b) In the obstetric population	Do non-criteria aPL exist and are they found in women with RPL?	Based on clinical studies = yes
	Are there sufficient clinical data to warrant a change in the 2006 Classification criteria	Not at the moment; more conclusive clinical studies are needed
	Do women with RPL who have early pregnancy losses and no thrombosis constitute a unique subgroup of APS with different diagnostic criteria of APS?	Obstetric populations should be stratified (with or without prior thrombosis and third-trimester losses from first trimester)

1b. Anti-domain I antibodies, IgA aCL and anti-β_2GPI antibodies, anti-prothrombin, and anti-prothrombin-phosphatidylserine antibodies

Test	Questions addressed by task force	Recommendations
Anti-domain I antibodies	Does the anti-domain I antibodies test recognize 'pathogenic' anti-β_2GPI antibodies?	Clinical data available encouraging
	Is there convincing evidence to include this test in the diagnostic algorithm of APS?	In vivo data with anti-domain I antibodies needed. Standardized consensus protocol for this assay needed Additional clinical studies needed
IgA aCL and IgA anti-β_2GPI antibodies	Are IgA aPL (particularly IgA anti-β_2GPI) clinically significant in patients with clinical manifestations of APS?	IgA anti-β_2GPI antibodies should be tested in the presence of clinical signs and symptoms of SLE and/or APS, particularly when other aPL tests are negative Evaluation and comparison of multiple, commercially available IgA aPL assays in A larger and well-characterized population of patients needed to confirm the diagnostic value of isolated anti-β_2GPI positivity Studies needed to determine the role of IgA anti-β_2GPI antibodies in the pathogenesis of APS IgA anti-β_2GPI antibodies that bind to domains IV/V of β_2GPI might represent an important subgroup of clinically relevant aPL antibodies
Anti-prothrombin and anti-prothrombin-PS antibodies; antibodies to negatively charged phospholipids other than cardiolipin	What is the role of anti-prothrombin and anti-prothrombin/phosphatidylserine antibodies in APS? Are antibodies to negatively charged phospholipids other than cardiolipin important in the diagnosis of APS?	aPT-A test in conjunction with other tests may be a good risk marker for thrombosis aPT-A and particularly the anti-PS/PT are good specific tests to confirm APS aPT-A and anti-PS/PT not ready to be included in the diagnostic criteria (standardization of the tests needed) Collaborative studies needed to confirm clinical associations with these tests
Annexin A5 (A5R) resistance test; anti-prothrombin and anti-prothrombin-PS antibodies	What is the role of the Annexin A5 resistance test in the diagnosis of APS? What is the role of anti-prothrombin and anti-prothrombin/phosphatidylserine antibodies in APS?	Data on the utility of AnxA5 resistance assay as a mechanistic diagnostic marker for APS are highly promising Developing mechanistic clinical assays that measure APS disease mechanisms is an important and appropriate avenue to pursue Additional data are needed before recommending A5R as a standard component of aPL testing panels

assay can be added to the official diagnostic guidelines. This assay needs to be made available to other centers for testing before any recommendation can be made (Table 1b).

Antibodies to negatively charged phospholipids other than cardiolipin: perspectives and experiences from a large reference laboratory in the USA (*Presented by Dr Tebo*)

Introduction and questions addressed by the task force

Antibodies directed against negatively charged phospholipids such as phosphatidic acid (PA), phosphatidylinositol (PI), and phosphatidylserine (PS) have been reported in patients with APS. However, the use of these antibodies in addition to the currently recommended laboratory markers for the diagnosis of APS remains controversial. Some investigators have suggested that testing for these aPL antibodies may help to identify women with recurrent pregnancy loss (RPL) with clinical features of APS who may benefit from treatment, a topic discussed in detail in the next section.[22–26] In other such studies, as well as in the context of thrombosis associated with systemic lupus erythematosus, no improvement in the diagnosis performance was observed when these were measured simultaneously with aCL and LA,[23–25,27] Therefore, these assays were not included in the 2006 revised criteria for the classification of APS.[1] In a review of the literature since the laboratory criteria for APS were revised, very few studies have been carried out to examine the relevance for these antibody markers. As such, most of the discussion and recommendations in this article will focus on the few recent investigations on this topic, with reference to some earlier key findings.

Early investigations by Gharavi and colleagues showed that aCL antibodies broadly cross-react to both antiphosphatidylserine (anti-PS) and antiphosphatidylinositol (anti-PI) antibodies.[28] Of the three major negatively charged aPL antibodies (anti-PA, anti-PI, and anti-PS), anti-PS has been most extensively investigated in thrombosis- and pregnancy-related morbidity APS.[22–27,29,30] These antibodies, particularly anti-PS, have been shown to be more specific for APS when compared with aCL, since aCL is often found to be positive in infectious diseases and other disorders.[31,32] However, the conditions necessary to achieve optimal clinical and analytical performance in these assays are yet to be determined.[1,29] Using aPS assays from two different manufacturers, Tebo et al. could not document a consistent diagnostic utility for this marker for both the IgG and IgM isotypes.[29] In addition, the combined use of these 'non-criteria' aPL antibodies differed significantly between manufacturers, especially for IgM specificities, and their overall combined diagnostic performance was not significantly higher than that of aCL and anti-β_2GPI assays.[29,30] Of clinical importance, no difference in the magnitude and prevalence of these antibodies was documented between healthy controls and women with recurrent pregnancy loss.[30]

Recommendations of the task force

In the evaluation of additional diagnostic markers for APS:

(a) It is important to determine critically whether, indeed, these antibodies contribute to the identification of additional patients who would otherwise be missed by the current assays or, alternatively, they would be better predictors of disease due to improved analytical and clinical performance. Anti-PA, anti-PI, and anti-PS antibodies in their current format pose significant diagnostic and analytical challenges. First, when they occur, they do so in high association with aCL antibodies and in isolation, and their clinical relevance is questionable and has not been fully investigated.
(b) In the case of anti-PS antibodies, the conditions required to detect this antibody remain controversial. Even for assays using the same reagents, the results are discordant as there are no formal calibrators or agreed methods of detection. Thus, in addition to not being cost-effective, to choose assays with the best medical benefit rather than a collection of tests with overlapping properties and equivalent or questionable clinical value may be the best practice.
(c) Based on the current evidence, it would appear that testing for anti-PA, anti-PI, and anti-PS antibodies in the initial diagnostic work-up for APS is not clinically useful, as these antibodies may have overlapping properties with the markers considered diagnostic for this disease.
(d) It would appear that the anti-PS marker may be the best candidate for further investigation of its relevance and significance, especially in the area of recurrent pregnancy loss, provided an accepted and standardized method is in place. In this case, more prospective studies using an agreed-upon protocol for patient recruitment,

follow-up, and testing for the presence of these antibodies are critical (Table 1a).

Antiphospholipid antibodies other than anticardiolipin antibodies in obstetric APS

(Presented by Dr Kutteh)

Introduction

Several investigators worldwide have advocated the use of a panel of aPL antibodies (aPL) to screen for APS.[33,34] This panel of tests includes not only cardiolipin (CL, diphosphatidyl glycerol) but also phosphatidyl inositol, phosphatidyl glycerol, phosphatidyl serine, and other negatively charged phospholipids. These phospholipids are found in various proportions on virtually every cell in the body, on the inner and outer surface membranes. Controversy has arisen as to the significance of these antibodies and whether treatment should be based solely on positive results of aCL or on positive results of any other aPL.

This ongoing debate of the clinical significance of aCL and other aPL has prompted some clinicians to screen recurrent pregnancy loss (RPL) patients and indentify those that might be missed if only aCL were considered significant. For example, Branch et al. analyzed the 95th and the 99th percentiles of the positive and negative cut-off for a panel of phospholipids among 147 women with RPL, APS, and fertile controls.[23] By using the 99th percentile, they found that 26/147 (17.7%) of women with RPL had positive antibodies to CL and 13/147 (8.8%) with RPL demonstrated binding against phospholipids other than CL or lupus anticoagulant (LA). The cut-off value in phospholipid units was determined by using the 99th percentile of the normal population, approximately threefold the median value. Based on comparison with controls, they concluded that this difference was not clinically significant.

In a much larger, earlier study, Yetman and Kutteh determined the prevalence of aPL among 866 women with RPL 5. In this population, 150 of 866 (17.3%) women with RPL were positive for IgG and/or IgM aCL while only 12 of 288 (4%) of control women without a history of poor obstetrical outcome were positive for the same antibodies ($p < 0.001$). The same study identified 87 of 866 women with RPL who were negative for aCL but positive for one of the other aPL, considering patients with more than one positive aPL only once.[35] Although this study was retrospective, it suggests that a significant number of women with RPL would not have been identified if they had been tested solely for aCL. The same group recently reported on another group of 872 women with RPL.[36] Positive aCL were detected in 132 of 872 women with RPL (15.1%), LA was detected in 31 of 872 (3.6%), and aPS was identified in 49 of 872 (5.6%) of women with RPL who were negative for aCL and LA.[36] Anti-PS antibodies were found in the absence of aCL and LA in women with RPL and two consecutive losses (18/391 or 4.6%), women with three consecutive losses (16/288 or 5.6%), and women with four or more consecutive losses (15/193 or 7.8%). In control women without a history of poor obstetric outcome, positive aCL were detected in 4.9%, positive LA in 1.0%, and positive aPS in 2.8%. Differences in aCL and anti-PS when comparing women with RPL to controls were significant using the two-tailed Fisher exact test.

The lack of standardization among different laboratories has made it difficult for physicians to identify patients with APS and those at risk for a miscarriage.[37–39] This has been used as a reason for not using other aPL as APS criteria, but in fact a great deal of variation exists between laboratories even when assaying aCL. For example, IgG aCL, considered by almost all clinicians and laboratory professionals as the 'gold standard', is still not standardized to the level of uniform agreement in all labs and all assays. In 2009, the College of American Pathologists survey results for sample ACL-06 showed that only 78% of labs could even agree that the sample was positive, while 5.5% of the labs determined the sample was negative, and the remaining 16.5% of the labs indicated that the result was indeterminate! Thus, an international group of investigators has established both clinical and laboratory criteria for the diagnosis of APS.[1] Yet, problems still exist when pregnancy loss patients are referred to fertility clinics that may have had testing performed at different laboratories using different control values and cut-off values to determine positive results. Also, standard testing may exclude a population of aPL patients who have had significant obstetric problems but test positive for other aPL and negative for the most commonly assayed aCL and LA.

Basic science supports the significance of aPL other than aCL. Anti-PS antibodies have been shown to inhibit trophoblast development and invasion using an in vitro model system.[40] Anti-PS retard syncytiotrophoblast formation and

decrease the synthesis of hCG. Both low-molecular weight and unfractionated heparin have been shown to reduce the in vitro binding of anti-PS as well as aCL.[41] Furthermore, some clinical data have been published suggesting that some women with a diagnosis of RPL and aPL positivity may benefit from treatments that have assisted women with RPL and aCL to deliver healthy offspring.[26]

Questions and answers from the task force

1. *Do non-criteria aPL exist and are they found in women with RPL?*

The task force generally felt that enough studies had been performed on large populations of patients to demonstrate that these 'non-criteria' aPL do indeed exist.[23,33–35]

2. *Are there sufficient clinical data to warrant a change to the 2006 criteria for the diagnosis of APS?*

The task force acknowledged that several studies have suggested that 'non-criteria' aPL may have clinical significance, but that the current level of evidence did not warrant any changes to the current criteria. Obviously, the task force would like to see more prospective, randomized trials, but acknowledged that a number of obstacles exist to make these types of studies difficult. These challenges include both clinical and laboratory inclusion criteria and the need to use an experienced laboratory in a multicenter study.

3. *Do women with recurrent pregnancy loss who have predominantly early pregnancy losses (prior to ten gestational weeks) and no history of thrombosis constitute a unique population that warrants different diagnostic criteria to APS?*

Considerable discussion on this topic was generated. It was felt that obstetric populations should be stratified to distinguish women based on their history of prior thrombotic events from those without this history. It was also felt that women with predominantly later-trimester losses (beyond 13 gestational weeks) should be distinguished from those women who had losses that were predominantly in the first trimester.[42] This population of women with early pregnancy losses may be affected differently by the non-criteria aPL through mechanisms other than thrombosis.[40] The task force felt that this should receive strong consideration at the next consensus conference.

Recommendations of the task force

The 'non-criteria' aPL task force agreed that studies from several different investigators clearly demonstrate that there are women with RPL who are negative when tested for aCL and LA but who are positive for other 'non-criteria' aPL. In fact, some of the task force members reiterated previous suggestions that women with RPL without a history of thrombosis should be placed in a separate classification when considering the diagnosis of APS, and that a treatment algorithm be constructed to address this group. However, the task force is uncertain and unwilling at this time to make any changes in the current criteria for the diagnosis of APS. It was agreed that some clinical studies show promise and need to be repeated by other groups, as those available do not have enough power to be considered significant. The task force felt that the significance of a panel of aPL antibodies to diagnose APS is an ongoing debate, with many complex questions that can only be addressed with larger study groups using an experienced central laboratory and multiple sites (Table 1a)

IgA anticardiolipin (aCL) and IgA anti-β_2GPI antibodies

(Presented by Dr Murthy on behalf of Dr Pierangeli's group and by Dr Petri)

Introduction and questions addressed by the task force

The current laboratory criteria for APS include the presence of positive lupus anticoagulant (LA) and/or IgG or IgM isotypes of aCL and/or anti-β2GPI antibodies, but omit the IgA isotypes for both tests.[1]

a) IgA aCL antibodies

Studies have shown data on the prevalence and significance of IgA aCL antibodies. In unselected patients with systemic lupus erythematosus (SLE), the prevalence of increased titers of IgA aCL has been reported to vary from 1% to 44%.[43–51] The lowest reported frequency was that found by Selva-O'Callaghan et al., who detected IgA aCL in only 2 of their 200 (1%) patients with SLE.[52] Alarcon-Segovia et al., in an earlier study that included 500 patients with SLE, found increased titers of IgA aCL in 16.6% of their patients.[53] In another study, Spadaro et al. found that IgA aCL was positive in 13 (20%) of their 65 SLE patients.[54]

In contrast, Weidmann et al. found IgA aCL to be positive in 44% of 92 SLE patients and also found IgA to be the most frequent aCL isotype.[45] The reported frequency for raised IgA aCL was higher (52.5%) in an earlier study by Wilson et al., where patients were preselected for being IgG or IgM aCL positive and/or having APS-associated clinical complications.[46] A prevalence of 83.3% was reported by Lopez et al. in a group of patients with SLE and thrombocytopenia.[47] As noted, the ethnic group composition of patients can influence the isotypic distribution of aCL. Molina et al. studied African-American, Afro-Caribbean, and Hispanic patients with SLE and found elevated levels of IgA aCL in 16%, 21%, and 14%, respectively.[48] The most important finding was that IgA aCL was the only aCL isotype present in 82% of aCL-positive Afro-Caribbean patients. In contrast, IgA aCL was found to be positive only in 4.4% of Chinese patients with SLE.[49] In another study, Cucurull et al. found that, although IgA aCL antibodies were present in 51% to 55% of patients with APS, most were also IgG or IgM positive, suggesting that measurement of IgA aCL would add little to IgG and IgM determination.[50]

There is some experimental evidence that IgA aCL antibodies are pathogenic. In a mouse model designed to study thrombus formation, injected IgA immunoglobulins with aCL activity from patients with APS were shown to cause thrombosis. The mean thrombus size using two different IgA immunoglobulin preparations was found to be significantly larger compared with control IgA.[55]

Numerous studies have also investigated possible associations between raised levels of aCL and clinical manifestations of APS attributed to these autoantibodies. Several of these studies reported a significant association for IgA aCL with one or more of the main clinical manifestations of APS. Cucurull et al., studying both aCL and anti-β2GPI antibodies in African-American patients with SLE, found an association between thrombotic events and raised levels of both these autoantibodies.[50] However, the number of their patients with thrombotic events was very small: only 5% of their 100 patients had documented evidence of thrombosis.[50] An association between raised IgA aCL levels and thrombocytopenia in patients with SLE or other collagen vascular diseases has also been reported.[56] Finally, an association between IgA aCL and recurrent fetal loss and with unexplained spontaneous abortions has been reported in women with SLE.[57] In a study that tested over 700 samples from an APS registry (APSCORE), only five samples were positive for IgA aCL alone and four of those were from patients who had presented with at least one of the two major manifestations of APS, according to the Sapporo revised criteria (unpublished observations). Furthermore, although the number of APS patients with IgA aCL positive results only – in the absence of IgG and/or IgM aCL-positive results – is low, its presence seems to be associated with clinical manifestations for the APS.[57] At this preconference workshop, Dr Michelle Petri showed data from her own laboratory, indicating that isolated IgA aCL positivity is rare but is associated with venous and arterial thrombosis.

b) IgA anti-β_2GPI antibodies

Previous studies have raised the possibility that IgA anti-β_2GPI might be associated with clinical manifestations of APS; those observations showed that SLE patients with APS are more prone to be positive for the IgA isotypes.[58–61] Furthermore, it seems that IgA anti-β_2GPI antibodies are independent risk factors of acute myocardial infarction and atherosclerotic disease in populations without APS (OR 3.4, CI 1.3–9.1),[62] and the same positive association was found for acute cerebral ischemia.[63–66] A concise report by Yamada et al. also showed anti-β_2GPI positivity in the absence of IgG anti-β_2GPI in a subgroup of women with unexplained recurrent pregnancy loss (particularly in the first trimester).[67] Similar findings were reported by Lee et al., indicating that IgA anti-β_2GPI positivity is more common in women who experience unexplained recurrent spontaneous abortion and unexplained fetal death and whose initial test results for other isotypes and LA were negative.[68] Further characterization of IgA anti-β_2GPI positivity in the absence of IgG anti-β_2GPI positivity associated with vascular morbidity showed that these antibodies may recognize domain IV of β_2GPI as their epitope.[69,70] In patients with SLE, the IgA anti-β_2GPI that recognizes domains IV and V seems to be positively correlated with thrombosis.[69–71]

Recently, Kumar et al. (from Dr Pierangeli's group) reported five isolated cases of individuals who were *exclusively* positive for IgA anti-β_2GPI and had concomitant clinical manifestations of APS.[72] Subsequently, Sweiss et al. reported that the presence of isolated IgA anti-β_2GPI positivity is associated with an increase in thromboembolic events, especially among patients with SLE. In that study – which included only a small group of SLE patients – IgA anti-β_2GPI was associated with an increased prevalence of morbidities involving

organs of mucosal immunity.[73] IgA anti-β_2GPI-isolated positivity has also been reported in both scleroderma and autoimmune hepatitis, and it was shown to correlate with both disease severity and endothelial damage.[74,75]

This task force further addressed the question whether IgA anti-β_2GPI may have diagnostic value for APS. First, the task force asked attendees of the 13th International Congress on APL antibodies to fill in a survey questionnaire on the use of IgA anti-β_2GPI assays. Thirty responses were returned and, of those who responded, 47% indicated that they routinely order or perform IgA anti-β_2GPI tests in their units; 25% indicated that they find an unusual number of patients with isolated IgA anti-β_2GPI tests; and 83% responded that those isolated IgA anti-β_2GPI are associated with manifestations of APS. Sixty-three percent of the responses indicated that a higher incidence of isolated IgA anti β_2GPI is seen in patients with SLE. Finally, approximately 44% of the responses indicated that IgA anti-β_2GPI tests should be used in confirmation of the diagnosis of APS.

Second, a group of investigators from Dr Pierangeli's laboratory presented data from a recent study where they examined the prevalence of isolated IgA anti-β_2GPI in 588 subjects with SLE from a large, multi-ethnic, multicenter cohort, Lupus in Minorities: Nature vs nurture (LUMINA), in 200 sera from SLE samples provided by Drs Akhther and Petri, and also in the sera of 5098 individuals referred to Dr Pierangeli's reference clinical laboratory (APLS) for APS work-up between January 2008 and March 2010 and correlated with the presence of APS-related clinical manifestations. The data were presented at this preconference workshop by Dr Murthy. aCL antibodies (IgG, IgM, IgA isotypes) and IgA anti-β_2GPI antibodies were evaluated by ELISA. IgA anti-β_2GPI titers were determined in two commercial FDA-cleared ELISA kits (kits 1 and 2). The binding of the IgA anti-β_2GPI-positive sera to domains IV/V of IgA anti-β_2GPI was also examined by ELISA. A total of 149 patients were found to be positive for IgA anti-β_2GPI isotype – 80 from LUMINA, 34 from Dr. Petri's cohort, and 35 from the APLS cohort. Of these, 35 from the LUMINA study, 15 from the Petri cohort, and 25 from the APLS cohort were found to be *exclusively* positive for the anti-β_2GPI isotype while being negative for the other aPL antibodies, including IgA aCL.[70] A significant number of subjects in the three groups had at least one APS-related clinical manifestation (70% in LUMINA, 100% in the Petri cohort, and 80% in the APLS group). These manifestations included: venous and arterial thrombosis (i.e., deep vein thrombosis, strokes, myocardial infarction); transient ischemic attacks; thrombocytopenia; miscarriages; and other symptoms such as livedo reticularis, pulmonary hypertension, cognitive dysfunction, and seizures. In kits 1 and 2, 86% and 85%, respectively, of IgA anti-β_2GPI were found to be positive. All samples were positive for IgA anti-β_2GPI in at least one kit. The correlation between the two kits was found to be 0.93.

In addition, 55% of the IgA anti-β_2GPI-positive sera (LUMINA and APLS cohorts) reacted with domains IV/V of the β2GPI, and 77% of those had clinical manifestations of APS that included deep vein thrombosis, strokes, myocardial infarction, pulmonary hypertension, seizures, pregnancy losses, skin ulcers, and livedo reticularis

In summary, Pierangeli and collaborators showed that a significant proportion of subjects in three different cohorts were positive solely for IgA anti-β_2GPI, and many of these had clinical manifestations of APS.[76] Their data confirm that isolated IgA anti-β_2GPI antibody titers may identify additional patients who have clinical features of APS but who do not meet current diagnostic criteria. We also concluded that IgA anti-β_2GPI antibodies that bind to domains IV/V of β_2GPI might represent an important subgroup of clinically relevant aPL antibodies.

Dr Petri also presented data at this preconference workshop proving that anti-β_2GPI of the IgA isotype is associated with thrombosis in SLE patients.[77] In her studies, IgA anti-β_2GPI was found in 10.2% of SLE patients, and as the sole anti-β_2GPI isotype in 13.1%. The association of IgA anti-β_2GPI antibodies with APS manifestations is shown in Table 1. The IgA anti-β_2GPI antibody was more strongly associated with deep venous thrombosis than the IgM isotype.[77] Second, the specificity of the association was also shown in those with IgA anti-β_2GPI alone: 22.1% had venous thrombosis and 11.9% had arterial thrombosis.[69,77]

Interestingly, discrepant results and significant lack of concordance among different IgA aCL and IgA anti-β_2GPI assays were obtained during a wet workshop at APLA 2010, when 26 APS samples were tested simultaneously in six different commercial IgA aCL and anti-β_2GPI assays, indicating that there may be substantial differences in the performance of various IgA assays.

Recommendations by the task force

a) IgA aCL

IgA aCL antibodies appear to be similar to IgG aCL in terms of thrombogenicity and cofactor requirement. Controversies regarding their prevalence and clinical associations still exist, perhaps due to the use of various nonstandardized assays and from differences in the design of the studies. Because of the very small prevalence of IgA aCL positivity alone in the absence of IgG and/or IgM aCL positivity, IgA aCL testing should be recommended in cases where IgG and IgM aCL are negative and there is a strong suspicion of APS.

b) IgA anti-β_2GPI

Based on the published evidence available (April 2010) – thoroughly reviewed by this group – and the studies presented by members of the task force at the preconference workshop at the 13th International Congress on Antiphospholipid Antibodies (APLA 2010), IgA anti-β2GPI antibodies should be tested in the presence of clinical signs and symptoms of SLE and/or APS, particularly when other aPL tests are negative. The group also recognized that well-designed studies, which should include evaluation and comparison of multiple commercially available assays in larger and well-characterized populations of patients, are needed in order to confirm the diagnostic value of isolated anti-β2GPI positivity before this test can be included in the diagnostic criteria of APS. The group also recommended that investigation should be carried out to determine the role of IgA anti-β2GPI antibodies in the pathogenesis of APS (Table 1b)

Antiprothrombin antibodies: aPT-A and aPS-PT

(Presented by Drs Bertolaccini, Forastiero, Binder, and Atsumi)

Introduction and questions addressed by the task force

The presence of antibodies solely targeting human prothrombin (aPT-A) by enzyme-linked immunosorbent assay (ELISA) has been recognized since 1995.[78] Several ELISA methods have been reported,[79–84] most of which use irradiated plates and buffers containing detergent (Tween 20), but the use of non-gamma-irradiated plates has also been proposed. The presence of Tween in the washing buffer enhances the binding of antibodies to the antigen, and this effect was found in both irradiated and nonirradiated microtiter plates. There is an ample variety of commercial microtiter plates and diverse blocking solutions used by different researchers. A major problem is that several in-house methods do not evaluate binding to empty or blank wells of each serum sample in order to assess nonspecific binding. The use of an irrelevant protein such as bovine serum albumin instead of only buffer for coating the control wells improves the performance of the aPT-A assay.[84] Several methodologic variations were assessed in an attempt to optimize the aPT-A assay:[85] the combination of gamma-irradiated plates, phosphate-buffered saline buffer, and a coating antigen of 10 μg/ml prothrombin was found the most sensitive. In recent years, a number of commercial kits for the detection of aPT-A have been made available. In a collaborative study assessing different in-house and commercial anti-PT assays, a good interassay concordance was found for IgG aPT-A using in-house and commercial kits, while IgM results were discordant between assays.[86]

Anti-PT antibodies bind not only to prothrombin coated on gamma-irradiated or -activated polyvinyl chloride ELISA plates (aPT-A), but also recognize prothrombin exposed to immobilized phosphatidylserine (phospatidylserine-dependent antiprothrombin antibodies, anti-PS/PT).[81] Antiprothrombin antibodies have been detected against prothrombin-bound, hexagonal (II)-phase phosphatidylethanolamine,[87] but this finding has not been fully investigated.

Although aPT-A and/or aPS-PT are associated with APS-related clinical features and these antibodies correlate with each other, aPT-A and aPS-PT belong to different populations of autoantibodies, even though they can both be present in the same patient.[88]

A number of studies have been published with regard to the relationship between APS-related clinical features and the presence of aPT-A, with conflicting conclusions.[79–82] High levels of aPT-A were found to confer a high risk of myocardial infarction in dyslipidemic middle-aged men without autoimmune disease.[81] Although no association between aPT-A and the risk of thrombosis was found in a systematic review,[89] there are some data suggesting that aPT-A are likely a risk factor of recurrent venous thromboembolism.[90] The majority of these studies were retrospective, and this fact makes it difficult to draw definite conclusions.[84,85,91–93] In recent years at least two

prospective studies have shown for the first time that the presence of aPT-A is a predictor of first or recurrent thrombosis in aPL patients.[94,95] The results of a 15-year longitudinal study showed that IgG aPT-A is the most useful predictor of thrombosis in SLE patients.[95] In addition, an important observation reported by several recent studies is that the risk of thrombosis progressively increases with the number of positive aPL tests. The quadruple positivity of lupus anticoagulant, aCL, anti-β_2GPI antibodies, and aPT-A seems to confer the highest risk for thrombosis.[96]

Many reports have also shown the clinical utility of anti-PS/PT assay for the diagnosis of APS.[88] Galli et al.[89] showed aPS-PT in 95% of their patients with thrombosis, but no differences in prevalence were found between those patients with thrombosis and those without. Funke et al.[97] reported that aPS-PT conferred an odds ratio of 2.8 : 1 for venous thrombosis and of 4.1 : 1 for arterial thrombosis in patients with SLE. Atsumi et al.[93] supported these data by showing that the presence of aPS-PT conferred an odds ratio of 3.6 : 1 for APS in 265 Japanese patients with systemic autoimmune diseases. Bertolaccini et al.[88] confirmed the association between aPS-PT (IgG and/or IgM isotype) and arterial and/or venous thrombosis. Both sensitivity and specificity of aPS-PT for the diagnosis of APS have been shown to be higher than that of aCL. In addition, aPS-PT strongly correlates with the LA, also suggesting that anti-PS/PT may be one of the 'screening' or 'confirming' assays for APS-associated LA.[93,98]

Recommendations of the task force

Based on the evidence published in recent years, it appears that the detection of aPT-A in conjunction with the other aPL tests could be useful in the consideration of risk for thrombosis.

The task force members agreed that anti-PT antibody assay – in particular, anti-PS/PT – would potentially contribute to a better recognition of APS. However, the inclusion of anti-PT antibodies as one of the laboratory criteria of APS cannot be warranted at this time, mainly due to poor standardization of aPT-A and/or anti-PS/PT.

Reproducibility of such strong correlations between anti-PS/PT and APS manifestations, which were presented by some investigators,[99] should be confirmed by the collaboration design. A multicentre study was proposed during the workshop discussion, and is currently being designed by task force members (Table 1b)

The annexin A5 resistance test: a mechanistic test for the detection of pathogenic aPL antibodies

(Presented by Dr Rand)

Introduction and questions addressed by the task force

Dr Jacob Rand from the Montefiore Medical Center, New York presented data on the annexin A5 resistance (A5R) test. Dr. Rand provided the committee with a brief historic background on current aPL tests – the aPL immunoassays and the lupus anticoagulant assays – all of which were derived empirically and do not report on thrombogenic mechanisms. The Rand laboratory has developed a novel functional assay that measures a disease mechanism – aPL antibody-mediated disruption of an anticoagulant shield that is composed of annexin A5 (AnxA5). The assay is based on the concept that AnxA5 has potent anticoagulant properties that result from its forming 2-dimensional crystals over phospholipids, blocking the availability of the phospholipids for critical coagulation enzyme reactions.[100–102] Previous research over the past 17 years has yielded strong evidence that aPL antibodies can disrupt this anticoagulant shield and unmask thrombogenic anionic phospholipids, which may thereby contribute to thrombosis and pregnancy complications in patients with APS.[103–107] The A5R assay is a 2-stage coagulation assay that mimics this mechanism on phospholipid suspensions.[108–110] The assay measures the effect of patient plasma on the anticoagulant activity of AnxA5; results are reported as percentage prolongation of the coagulation time by AnxA5; patients with percentages lower than the reference range are considered to have AnxA5 resistance. Remarkably, resistance to AnxA5 anticoagulant activity has been correlated with aPL antibodies that recognize an epitope on domain I of β_2GPI.[109] Dr Rand provided details on the methodology and, with Dr Xiao-Xuan Wu, demonstrated the assay in the meeting's wet laboratory demonstration session. The assay is labour intensive and, as mentioned above, requires a 2-stage procedure in which the first stage exposes the phospholipid suspension to patient plasma, and the suspension is then centrifuged and washed for the second stage in which the phospholipid is used to coagulate a normal pooled plasma.

Dr Rand presented the task force with data collected from five studies on coded samples from

597 patients – all of which were obtained from collaborators at outside institutions. The available evidence strongly supports the utility of this mechanistic assay in defining a subgroup of patients in whom this disease mechanism occurs. The pooled data indicated that about half (52%) of patients with symptomatic APS by current consensus criteria have AnxA5 resistance, whereas 2–5% of disease-free controls and patients with non-APS thrombosis have that abnormality. Interestingly, 27% of patients who tested positive for aPL antibodies but did not have a history for thrombosis also tested positive for AnxA5 resistance. Since many of the latter were patients with autoimmune conditions such as SLE, Dr Rand hypothesized that these patients might have an increased risk for future thrombosis – a concept that would need to be validated in prospective longitudinal observational studies.

Recommendations of the task force

The task force committee concluded that data on the utility of AnxA5 resistance assay as a mechanistic diagnostic marker for APS are highly promising. The committee also felt that the concept of developing mechanistic clinical assays that measure APS disease mechanisms was an important and appropriate avenue to pursue. The committee would like to see additional data before recommending A5R as a standard component of aPL testing panels. In addition, the assay needs to be made available for other centers to be tested before any recommendation can be made (Table 1b).

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Conflict of interest statement

None declared.

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特集 不妊・不育
助産師に今，求められる知識とケア

2

妊娠を維持するメカニズムとその病態

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POINT

1. 不育症の原因について学びましょう！
2. 年齢と妊娠の関係について学びましょう！
3. 精神的支援の重要性を学びましょう！

はじめに

自分を責めないで
女性の仕事や運動によって流産が起こるという科学的根拠はありません。自分を責める必要はないことを言ってあげましょう。

流産は妊娠22週未満の娩出と定義されますが，妊娠10週未満の早期流産が大多数を占めます。超音波検査がなかった時代は妊娠の診断が現在よりかなり遅く，後期流産しか認識されていませんでした。「階段から落ちて流産する」というような場面をドラマなどでもしばしばみかけますが，このような外傷性後期流産はきわめてまれです。しかし一般の人は女性の労働，不摂生のために流産は起こると現在も思っているのでしょう。労働が原因で流産が起こるという科学的根拠はありません[1]。

流産は妊娠最大の合併症であり，約15％の妊婦に起こります。また女性の加齢とともに増加し，40代では50％にも上ります（図1）。

習慣流産は3回以上連続する流産，不育症は妊娠はするけれど流産・死産によって生児を得られない場合，と定義されています。私たちが実施した日本初の疫学研究岡崎コホート研究によれば，一般集団における習慣流産頻度は0.9%，不育症は4.2%，妊娠したことのある女性の38%が流産を経験していました[3]。

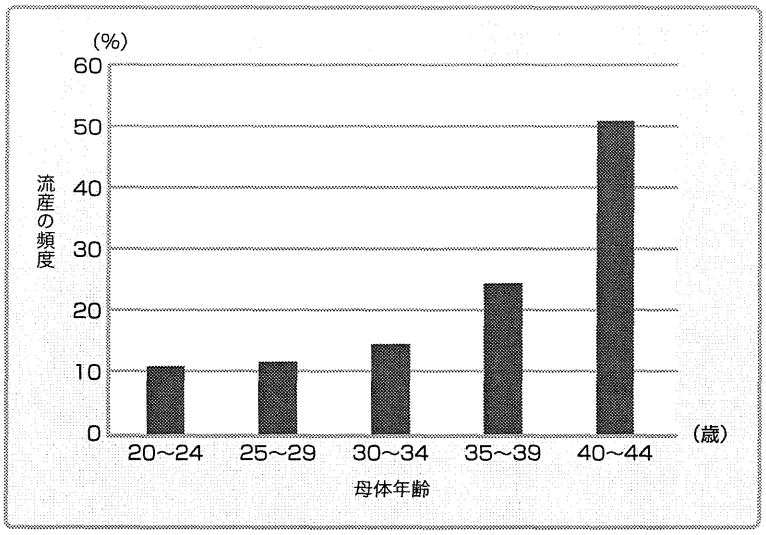

図1 加齢による流産の頻度[2]

早期流産と染色体異常

流産，とくに早期流産のほとんどは胎児（胎芽含む）の染色体異常によって起こります。

女性の卵は胎生20週頃に最大数の700万個になり，閉経までアポトーシスにより減少し続けます。個々の卵は第一減数分裂の途中で停止しており，排卵の時点で減数分裂を再開します。加齢によって，排卵時に各染色体の分配が正常に起きない不分離現象が起こりやすくなり，染色体数的異常を引き起こすことがあります。胎児の50～70%に染色体異常がみられると報告されていますが，私たちの検討では76%でした[4]。このばらつきは女性の平均年齢によるものです。

染色体異常の種類としては16番トリソミーが最も多く，45，Xを除くモノソミーは流産児にはみられません。染色体異常は発生早期ほど高率にみられ，モノソミーはより重篤なため，不妊となると推定されています。

妊娠を維持するメカニズムとその病態

妊娠の維持には成長因子やサイトカインが関与しています（図2）。白血病抑制因子（leukemia inhibitory factor；LIF）は子宮内膜，脱落膜から分泌され子宮内膜の脱落膜化と着床の両方に必須です。着床にはプロスタグランジン産生が必要であり，Cyclooxygenase-2が着床においてこれを制御しています。着床後は絨毛性ゴナドトロピンにより，黄体からプロゲステロンの産生が維持され，プロゲステロンにより，子宮内膜は脱落膜に変化します。脱落膜のVascular endothelial factorは血管新生に，interleikin-1，colony-stimulating factor 1，transforming growth factors α，βは絨毛の増殖に重要な役割を担っています。

絨毛表面にはHLA-Gが表出しており，ヒトの他の組織にみられるclass Ⅱの抗原は表出していません。胎児は母体にとって，半分は他人の抗原を持つ異物であり，これが免疫学的拒絶を受けない理由はHLA-Gにあると推定されています。子宮脱落膜にはナチュラルキラー細胞とマクロファージが多数存在しており，

これらはHLA-Gを認識したうえで胎児を拒絶することなくサイトカインを介して絨毛の発育を促進します。これが免疫学的寛容，すなわち免疫学的妊娠維持機構です。これらの一部に異常が生じれば流産となることが推定されます。

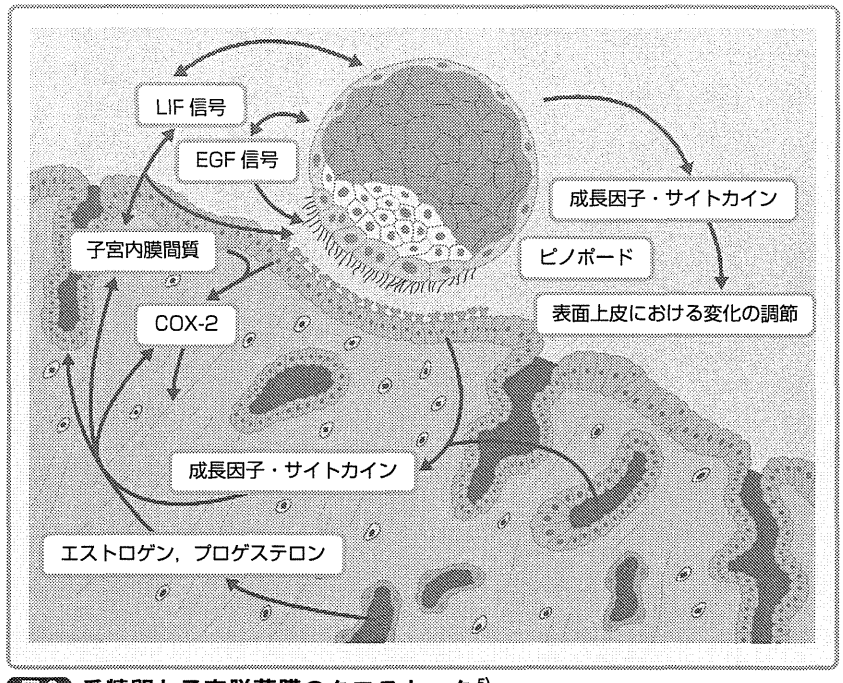

図2 受精卵と子宮脱落膜のクロストーク[5]

不育症原因精査のための検査

不育症の原因は，抗リン脂質抗体10%，子宮奇形3.2%，夫婦どちらかの染色体異常6%，糖尿病・甲状腺機能異常・多のう胞性卵巣症候群などの内分泌異常12%と考えられます（表1）。生殖内分泌異常，免疫異常，血栓性疾患，遺伝子異常，精神的ストレスなどの関与も報告されていますが，まだ研究段階です。

表1 不育症に必要な検査項目とその意義

	頻度	推奨検査		学会推奨レベル※2
抗リン脂質抗体	10.7%	β2glycoprotein I 依存性抗カルジオリピン抗体（230）※1 ループスアンチコアグラント（dRVVT 290）※1 ループスアンチコアグラント（希釈 aPTT 法）※1	陽性率は 2%程度，特異度は高い 陽性率は 2%程度，特異度は高い 陽性率は 15%，中和試験によって確認する 陽性の場合 12 週間後に再検査し，陽性が持続したら抗リン脂質抗体症候群と診断	A
子宮奇形	3.2%	子宮卵管造影（512）※1 と超音波検査（530）	双角子宮，中隔子宮，重複子宮，単角子宮は流産の原因 手術によって生児獲得率が改善するという証拠はまだ得られていない 弓状子宮は流産の原因とならない	A
夫婦染色体異常	6%	G 分染法（3130）	転座は流産の原因 9 番逆位は正常変異	B
内分泌異常	12%	糖尿病：空腹時血糖（11） 甲状腺機能異常：TSH（115），FT4（140） 多のう胞性卵巣症候群：超音波検査と月経異常の問診		C
凝固系検査		aPTT（29）	抗リン脂質抗体症候群では延長する 抗リン脂質抗体を測定するなら必要ない 妊娠中は短縮するためヘパリンのモニターとしては使えない	C
胎児染色体異常	51%	絨毛染色体 G 分染体	数的異常が多く，明らかな原因	C

※1 習慣流産として保険採用されている。スクリーニングとして検査する場合は保険採用できない。（2012年4月）
※2 日本産科婦人科学会ガイドライン推奨レベル

一方，散発性流産の約70％が胎児染色体数的異常によりますが，繰り返す流産はそのような偶然によるものではない，ということが長い間信じられてきました。しかし，不育症の集団にも胎児染色体異常を繰り返している症例が約51％存在することがわかってきました[4]。胎児染色体検査は行われないことが多く，ほとんどの論文で「不育症において半数以上が原因不明」と記載されています。しかし，胎児異常も原因として追究すれば真の原因不明は約20％になります（図3）。

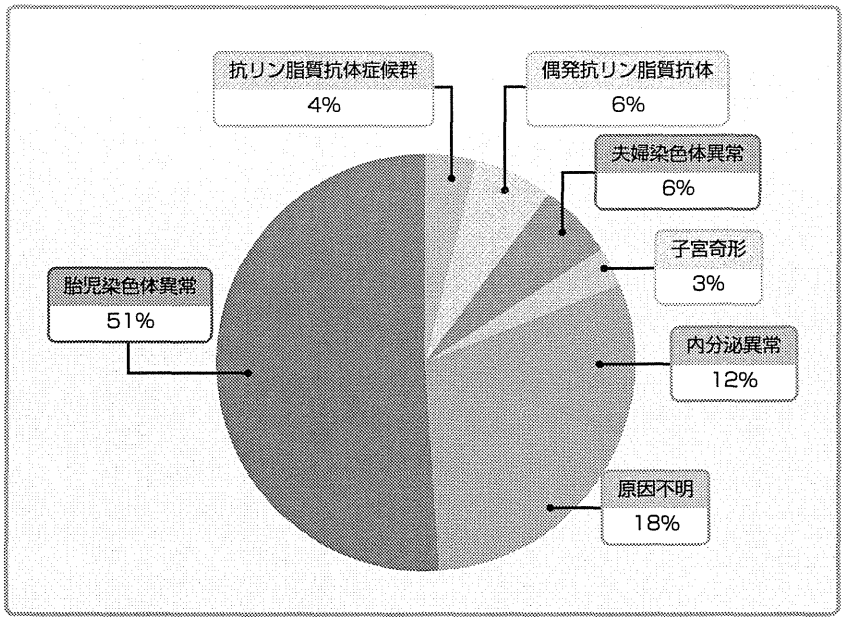

図3 名古屋市立大学の不育症患者1676組の異常頻度

抗リン脂質抗体症候群の診断方法

抗リン脂質抗体とは

抗リン脂質抗体の測定法にはリン脂質cardiolipin（CL），phosphatidylglycerol（PG），phosphatidylcholine（PC），phosphatidic acid（PA），phosphatidylinositol（PL），phospatidyethanolamine（PE）に対するIgG，IgA，IgMをELISA法で測定する場合と凝固時間を測定するLupus Anticoagulant（LA）があります。1990年には抗CL抗体の真の対応抗原はCLではなくβ2glycoprotein I（β2GPI）であることが判明しました。その後，血漿中のリン脂質結合蛋白であるProthrombin，kininogen（KN），Annexin V，Protein C，Protein Sなどが対応抗原として報告されましたが，現在もこれらを抗リン脂質抗体と呼んでいます。

表2 抗リン脂質抗体症候群診断基準[6]

臨床所見
動静脈血栓症
妊娠合併症 ◎妊娠10週未満の3回以上連続した原因不明な習慣流産 ◎妊娠10週以降の原因不明な子宮内胎児死亡 ◎妊娠34週未満の重症妊娠高血圧腎症・子癇や胎盤循環不全による早産
検査所見（12週間以上の間隔で2回以上陽性）
Lupus anticoagulant（LA）陽性（国際血栓止血学会ガイドラインに準じた方法）
（β2glycoprotein I依存性）抗カルジオリピン抗体IgGもしくはIgMが中高力価
抗β2glycoprotein I抗体IgGもしくはIgMが陽性

APS診断基準

抗リン脂質抗体症候群（anti-phospholipid syndrome；APS）診断基準を表2に示しました。反復流産，子宮内胎児発育遅延，子宮内胎児死亡，妊娠高血圧症候群，胎盤早期剥離，羊水過少などに抗リン脂質抗体陽性が疑われ，とくに早期流産よりも子宮内胎児死亡との関連が強いと考えられています。