Diplomarbeit

Antiphospholipid antibodies and pregnancy

eingereicht von

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zur Erlangung des akademischen Grades

Doktor(in) der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Lehrstuhl für Physiologie
Universitätsklinik für Frauenheilkunde und Geburtshilfe

unter der Anleitung von

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Graz am 28.05.2020

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, anders als die angegebenen Quellen nicht verwendet habe und die den benutzen Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 28.05.2020 *Julia Pailer eh*

Danksagung

An dieser Stelle möchte ich mich bei meiner Betreuerin Ao. Univ. Prof. Dr. Anna Gries bedanken, die immer ein offenes Ohr für mich hatte und mir mit Rat und Tat zur Seite stand. Ich danke auch Frau Univ-Ass. Dr.med.univ. Mayer-Pickel welche mich zusätzlich beim Verfassen dieser Diplomarbeit betreut hat. Weiters bedanke ich mich bei meiner Mutter. Seit meiner Geburt werde ich von ihr in all meinen Vorstellungen, Ideen und Taten unterstützt. Besonderer Dank gilt Professor Bernhard Ple welcher mir bei der Korrektur der Arbeit geholfen hat und meinem Mitbewohner Paul der mir bei dem Formatieren der Arbeit, sowie dem richtigen Umgang mit Zitierprogrammen geholfen hat. Schließlich bedanke ich mich noch bei meiner restlichen Familie, Freunden und StudienkollegInnen die mir meine Studienzeit „versüßt“ haben.

Zusammenfassung

Das Ziel dieser Diplomarbeit war es einen Überblick über die Definition und aktuelle Forschungsergebnisse über das Thema Antiphospholipid Antikörper im Zusammenhang mit Schwangerschaft bzw. die dadurch ausgelösten Komplikationen zu geben.

Interessant ist die Arbeit insbesondere für Personen im Medizin Studium oder Menschen, die im medizinischen Bereich tätig sind und ihr Wissen über das Antiphospholipid Syndrom vertiefen wollen.

Die Diplomarbeit bearbeitet den Zusammenhang zwischen Schwangerschaft und Antiphospholipidsyndrom. Weiters wird auf die Antiphospholipid Antikörper und Pathomechanismen, wodurch die Präsenz dieser Antikörper zu Problemen führt, eingegangen. Mögliche Komplikationen während der Schwangerschaft werden diskutiert. Abschließend werden Diagnose Kriterien und Therapie Guidelines beschrieben.

Anhand Literaturrecherche mittels Pub-Med wurden länger bestehende sowie aktuelle Arbeiten überprüft und zusammengefasst. In Hinsicht auf Definitionen wurden zusätzlich Lehrbücher verwendet.

Abstract

This thesis aims to give an overview of the definition and current research results on the topic of antiphospholipid antibodies in connection with pregnancy and the resulting complications.

This work is aimed at a wide audience from medical students to those working in medicine. Covering a wide range of subject matter including, antiphospholipid syndrome as well as pregnancy in general. Furthermore, the antiphospholipid antibodies and pathomechanism of the disease are discussed. Finally, the diagnosis criteria and therapy guidelines are listed.

Based on literature research using PubMed, existing and current works and papers have been studied, summarized and referenced within.

Material and Methods

In order to write this thesis, the first approach was reading three articles recommended by Professor Anna Gries. Those were the following:

1. “Antiphospholipid antibody profile based obstetric outcomes of primary antiphospholipid syndrome: the PREGNANTS study.” by Saccone G. et al., Am J Obstet Gynecol 2017, 525
2. “International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS).” by Miyakis S. et al., J Thromb Haemost 2006, 295-306
3. “Antiphospholipid antibodies and the risk of pregnancy complications” by Gris J. et al., Thromb Res 2017, S34-S37

Based on those, further research using the included lists of literature has been conducted, primarily focusing on papers including a general context in order to get an overview over the antiphospholipid syndrome and antiphospholipid antibodies. After studying those articles, the list of literature was used again to get a more specific insight into the topic.
After extensive research surrounding the antiphospholipid syndrome and related antibodies as well as complications, I chose a more detailed approach to form individual chapters. The following step was writing a table of content so that I was able to continue the writing of the thesis in a very organized way. This prevented rereading multiple papers or repeating already given information within the thesis.

In order to write chapter one, two books from the online library of the Medical University of Graz by “Springer Link” rather than medical articles have been used, since as an introductional chapter it includes general information that is best summarized in a book.

For the following chapters the electronic database “PubMed” has been used individually typing in the headlines or bullet points of the chapter followed by a more detailed search.

Inclusive criteria have been the following:

1. Date of publication
2. Best match
3. Title of the paper/article
4. Information included in the abstract

Exclusive criteria have been:

1. Repeated information of more popular works (those were identified by checking the table of references).
2. Outdated information

Furthermore the “Handbook of Systemic Autoimmune Disease” has been a central source for information regarding this thesis.

Key words, number of results within the search engine, number of papers found within the citation program Mendeley and numbers of papers used in the thesis are summarized in the following table:

Key words	No. of results	No. of papers used	No. of papers within Mendeley	No. of citations within the thesis
Antiphospholipid syndrome	11438	2	74	29
Antiphospholipid syndrome definition	328	2	6	2
Thrombotic antiphospholipid syndrome	5779	4	20	6
Obstetric antiphospholipid syndrome	1275	2	27	1
Antiphospholipid antibodies	12554	3	68	35
Antiphospholipid	710	3	5	6

antibodies laboratory detection				
Antiphospholipid syndrome pathogenesis	8999	3	25	32
Obstetric complications antiphospholipid syndrome	966	5	22	30
Plasmapheresis antiphospholipid syndrome	274	3	5	5
Antiphospholipid syndrome diagnosis	6422	10	18	8
Antiphospholipid syndrome treatment	5541	7	32	29
Obstetric antiphospholipid syndrome treatment	783	10	22	17

The chapter dealing with definition as well as epidemiology used the most frequent papers and definitions as they were standardized. In those subchapters 11 articles have been used.

To acquire information surrounding the history of APS the “Google” search engine has been used. When typing in the key word “history of antiphospholipid syndrome” 204.000 results can be found including a chapter from the “Handbook of Systemic Autoimmune Disease” which included a variety of useful information and a web page by Nigel E. Harris. Based on those and their included list of literature the chapter was formed using seven sources.

Papers and articles published by and after the 14th and 15th International Congress on APS the chapters dealing with diagnosis and treatment have been formed since they contain updated guidelines and verified information. Eight papers have been used for diagnosis and 29 for treatment.

The papers, articles and books that have been used are mostly in English, a small amount has been in German, Spanish or French.

For citation the program “Mendeley” has been used to sort articles and give further examples. 249 works have been included in total in this thesis.

Table of Abbreviations

ABI	Ankle-brachial index
aCL	Anticardiolipin
APA	Antiphospholipid antibodies
aPl	Antiphospholipid antibodies
APS	Antiphospholipid syndrome
aPTT	Partial thromboplastin time
aβ2-GPI	Anti-beta 2 glycoprotein I
BFP-STS	Biologically false positive serological test for syphilis
B2GPI	Beta 2 glycoprotein I
BMI	Body mass index
CAPS	Catastrophic antiphospholipid syndrome
CL	Cardiolipin
CSVT	Cerebral sinus vein thrombosis
D1	Domain one
dRVVT	Dilute Russel viper venom time
DTI	Direct thrombin inhibitors
ELISA	Enzyme-linked immunosorbent assay
EPL	Early pregnancy loss
EUROPAS	The European registry on obstetric Antiphospholipid syndrome
FD	Foetal death
FDA	Food and drugs administration
GFR	Glomerular fraction rate
GP	General practitioner
hCG	Human choriongonadotropin
HCQ	Hydroxychloroquine
HCS	Human chorionic somatomammotropin
HELLP- Syndrome	Haemolytic elevated liver enzymes low platelets syndrome
HMG-CoA	3-Hydroxy-3-methyl-glutaryl coenzyme A
hPL	Human placental lactogen
IFN–Y	Interferon -Y
INR	International normalized ratio
ISTH	International society on thrombosis and haemostasis
IUGR	Intrauterine growth restriction
LA	Lupus anticoagulant
LDA	Low dose aspirin
LMWH	Low molecular weight heparin
LPS	Lipopolysaccharides
mTOR	Mechanistic target of rapamycin inhibitors
NOAC	New generation anticoagulants
OAPS	Obstetric antiphospholipid syndrome

P.C.	Post conception
PAPS	Primary antiphospholipid syndrome
PAS	Perinatal arterial ischemic stroke
PE	Early severe preeclampsia
PI	Placental insufficiency
PT	Prothrombin
RDS	Respiratory distress syndrome
REPL	Recurrent early pregnancy loss
ROS	Reactive oxygen species
SAPS	Secondary antiphospholipid syndrome
SLE	Systemic lupus erythematosus
TF	Tissue factor
Th1	T helper cells 1
Th2	T helper cells 2
TLR	Toll-like receptors
TNF alpha	Tumour necrosis factor-alpha
VKA	Vitamin K antagonist

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1 Introduction

Antiphospholipid Syndrome is a disease affecting mainly the female population, the existence of antiphospholipid antibodies leads to a variety of complications including but not limited to thrombosis, recurrent early pregnancy loss, pre-eclampsia, HELLP-Syndrome and intrauterine foetal death. Extensive research has been carried out in the past investigating the pathomechanism, diagnosis and treatment of the disease. However, further research and more promising results must follow to shine light on this disease to prevent future pregnancy related complications (Levy RA. et al., 2015, p. 205).

This thesis aims to investigate the connection between antiphospholipid antibodies and pregnancy complications. Furthermore, it discusses the current diagnosis and treatment as well as treatment perspectives of the obstetric antiphospholipid syndrome.

1.1 Pregnancy

Fertilization, implantation, decidualization and placentation are complex. Each step is a precondition for the following and essential for a successful pregnancy outcome. In general, the terms fertility and fecundity must be distinguished. Fertility is the natural capability to produce offspring, whereas fecundity is a measurement for the success of fertility. Among the general population, the maximal fecundity lies among 30 % (Zinaman et al., 1996, p. 503) (Breckwoldt M. et al., 2000, pp. 287–305).

1.2 Fertilization and Implantation

The fertilization takes place between 24 and 48 hours after the ovulation in the fallopian tube of the woman. Four days after the conception (p.c.) the cell formation reaches the female uterus in the form of a blastocyst. Within the blastocyst, three components can be distinguished. An inner cell mass, the embryoblast, the blastocoel, a liquid-filled cavity and the trophoblast, an outer cell layer. The trophoblast will later form into the placenta and the blastocoel will rearrange itself into the amnion. The embryoblast will be divided into endo and ectoderm. Around the eighth- or ninth-day p.c. the implantation takes place (Breckwoldt M. et al., 2000, pp. 287–305).

1.3 Placentation

During the first days after fertilization, the various cell formations can absorb nutrients directly from the surrounding tube secretions and out of its ooplasm. In-further development, the placenta is formed and used to nourish the embryo and then the foetus. The placenta, as formerly mentioned, is formed out of the trophoblast and the maternal endometrium. The differentiation of the trophoblast, the decidualization and the foetal-maternal exchange of blood are three central components of the placental development. Decidualization is the reorganization of the endometrium into the decidua when coming into contact with the trophoblast. Furthermore, the formation of lacunae within the syncytiotrophoblast is crucial. Lacunae are cavities that come into direct contact with open maternal vessels, where the first exchange of blood can be observed. The lacunae then rearrange, forming a network that enables blood flow. The cytotrophoblast does not connect with maternal tissue. After further differentiation of the syncytiotrophoblast in between the lacunae, other masses, the trabeculae, can be observed. These are important for the physiological growth of the chorionic villi. Three consecutive stages of chorionic villi, primary, secondary and tertiary, can be distinguished. In the third stage, the villi form a cytotrophoblastic shell which surrounds the syncytiotrophoblast. The development of the tertiary chorionic villi lasts until the end of gestation. In between the cytotrophoblastic shell and the syncytiotrophoblast formed villi in the intervillous space, a liquid-filled cavum is formed (Breckwoldt M. et al., 2000, pp. 287–305).

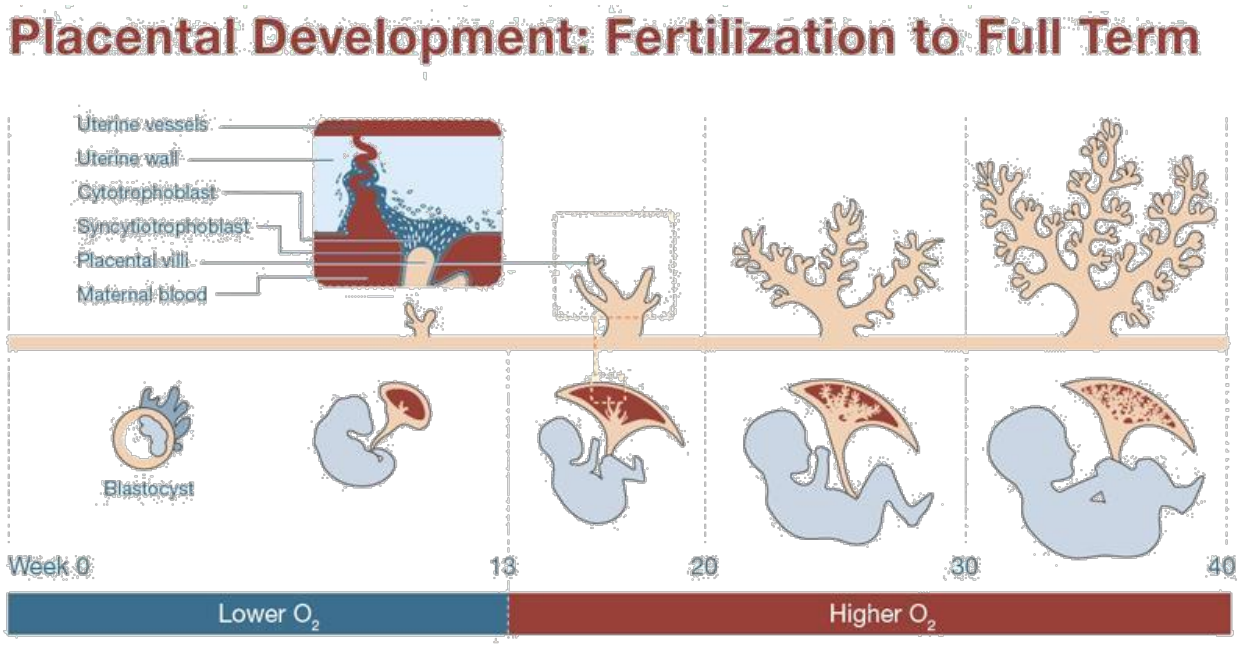

Figure 1: adapted from (Office of Communications, 2017) "Placental Development: Fertilization to Full Term"

2 Antiphospholipid Syndrome

2.1 Definition

The antiphospholipid syndrome is defined by the presence of antiphospholipid antibodies in the maternal circulation and the occurrence of arterial and/or venous thrombosis and pregnancy complications. Generally, three antiphospholipid antibodies (aPl) are related to APS: the anticardiolipin antibody (aCL), the anti-beta 2 glycoprotein I (aβ2-GPI) and the Lupus anticoagulant (LA). APS is defined through a combination of clinical and laboratory criteria. In general, two types of antiphospholipid syndrome are to be distinguished. The primary APS (PAPS), where affected patients only suffer from this single autoimmune disorder. On the other hand, secondary antiphospholipid syndrome (SAPS) occurs when people are affected by multiple autoimmune disorders. In most cases, Systemic lupus erythematosus or Lupus-like disease can be found additionally (Saccone G. *et al.,* 2017, p. 5). About 40 per cent of patients with systemic lupus erythematosus (SLE) will test positive for one of the above-stated antibodies (aCL, aβ2-GPI or LA) (Petri M., 2000, p. 146). Although extensive research has been made into the topic of the antiphospholipid syndrome the exact mechanism behind complications regarding pregnancy is yet to be further investigated.

2.2 History

In 1952 Conley and Hartmann (Conley and Hartman, 1952, p. 621) discovered the Lupus anticoagulant. Later Moore and Mohr (Keeling *et al.*, 2012, p. 47) revealed an association between LA and the Biological false test for Syphilis. Patients with a biologically false-positive serological test (BFP-STS) for syphilis were at the same time suffering from thrombosis (Harris E., 2009, p. 326). Conley and Hartmann wrote a brief report about two patients with SLE and a *"peculiar haemorrhagic disorder".* Both patients showed prolonged blood clotting times and high serum levels of LA in their blood (Conley and Hartman, 1952, p.621).

APS was first described by John Patterson Hughes and Peter George Ingle Stovin in 1959 (Hughes and Stovin, 1959, p.19). The Antiphospholipid Syndrome is sometimes also referred to as Hughes Syndrome.

In 1964 Walter Bowie and colleagues found out that the LA was not, as originally thought to cause haemorrhage but thrombosis. Later Johnson and Colleagues found out that an extensive number of patients with SLE also tested positive for LA (Cervera R., 2009, pp. 240–245). In 1975 Nelson and colleagues reported that the LA was also linked with intrauterine foetal death. Between 1980 and 1983 a lot of progress was made concerning APS and many reports were released (Harris E., 2009, p 326). Professor Hughes Graham and his team worked intensively to find a connection between LA, biologically false test for syphilis (BFP-STS), venous and arterial thrombosis and recurrent pregnancy loss. Additionally, they revealed that patients, suffering from thrombosis were negative for LA but positive for aCL. Around the year 1983 it was stated that patients positive for aCL are affected by thrombosis, recurrent pregnancy loss and thrombocytopenia.

In 1999 during the 8th International Congress of APS in Sapporo, a group of scientists created standardized criteria for APS in order to have regulated inclusive criteria for studies regarding this topic. However, nowadays they are used to aid with the diagnosis of the antiphospholipid syndrome. Included are signs and symptoms as well as criteria for laboratory testing. Those are single or multiple thrombotic events, regardless of the part of the body affected. Obstetric APS, including complications such as foetal death, occurring before or after 10 weeks of gestation, if genetic and/or hormonal aberrations have been eliminated as causing factors. Furthermore, the occurrence of either eclampsia, pre-eclampsia or placental insufficiency leading to a termination of gestation six or more weeks before the estimated date of birth. LA and aCL must be detected in laboratory testing in a sufficiently high amount at least two times with a six-week separation in between. In 2006 the criteria have been revised during the 11th International Congress of APS in Sydney. The included clinical and obstetric criteria have not been modified. However, regarding the laboratory verification of antiphospholipid antibodies aβ2GPI has been included. Furthermore, the time in between individual testing has been doubled. This lead to improved sensitivity due to the addition of aβ2GPI as well as higher specificity because of the longer period in between testing's (Miyakis S. et al., 2006, p. 295) (Weber, Hayem and Meyer, 2001, p 1965).

2.3 Epidemiology

At the moment, an exact number of individuals affected by APS cannot be stated, due to insufficient numbers of patients included in clinical studies, inadequate control groups and contrasting definitions of APS. It has been suggested that the most important factors are standardized features such as a sufficiently high number of patients. Furthermore, race, pregnancy status, gender and age should be subgroup divisions (Petri M. et al., 1987, p. 529). In a population of 100.000 people, the incidence would be at five new cases per year and the prevalence would be 40-50 cases (Gomez-Puerta and Cervera, 2014, p. 1). It is stated that women account for approximately 80 % of APS patients (Toh Y., 2019, p 264).

During each menstrual cycle, there is a 30 % chance of fertilization. 50 % of conceptions fail in later steps and about 10-12 % of the acknowledged pregnancies are not carried until term (Drakeley, Quenby and Farquharson, 1998, p. 1975). Of those unsuccessful attempts, the majority are idiopathic, which means that no causing factor can be established. On the other hand, between 0-50% are caused by the presence of aPl (Vinatier *et al.,* 2001, p. 39). This also indicates the importance of the association between APS and pregnancy. In 2017 a multicentre, retrospective cohort study was conducted. 173,842 deliveries from seven centres in Italy, occurring between January 2007 and April 2016, were investigated. 1201 (0,7%) women were previously diagnosed with PAPS. 750 patients of this cohort would have been included if they had met further criteria such as singleton pregnancies and if they had been examined in the first trimester (12 weeks). Patients with multiple pregnancies were excluded. The results of the study showed that 458 pregnancies (61%) were positive for only aCL. 128 pregnancies (17,1%) tested positive for only aβ$_2$GPI and 54 pregnancies (7,2%) positive for only LA. Therefore, in total 640 women were tested positive for only one of the three most common antibodies. On the other hand, the combination of multiple antibodies was also detected. The results show that 90 pregnancies (12%) were positive for aCL and aβ2-GPI but negative for LA. Twenty pregnancies (2,7%) were positive for all three antibodies. So in total 110 out of 750 women tested positive for multiple antibodies (Saccone G. *et al,.* 2017, p. 3).

Several studies have claimed that thrombosis is more likely to appear within an elderly demographic. This is the reason why APS is often diagnosed within this population group (Petri M., 2000b, p. 145). Individuals suffering from chronic illness are more likely to be diagnosed with APS. This is also a result of the extended tests and examinations of this population group (Juby and Davis, 1998, p. 4). About 50 % of individuals suffering from SLE and positive LA will present a thrombotic event in the following 20 years (Somers, Magder and Petri, 2002, p. 2535). LA solely is the antibody most likely leading to a thrombotic event (Ruffatti *et al.,* 2011, p. 1085). Single aCL and aβ_2GPI positive individuals are less likely to suffer from an episode. The development of arterial and/or venous thrombosis is often associated with “triple positive” antibodies. The group of patients has a 9,2 % risk after two years, and a 37 % risk after ten years (Pengo V. *et al.*, 2011, p. 4716). As expected, the risk was decreased when a prophylactic medication was given.

2.4 Types of APS

In general, primary and secondary antiphospholipid syndrome are distinguished. Furthermore, there is a differentiation between the thrombotic APS (TAPS) and Obstetric APS (OAPS), which is linked in detail to obstetric difficulties. CAPS is a rare life-threatening form of APS, characterized by dissemmated intravascular thrombosis resulting in multiorgan failure (Levine and Branch, 2002, p. 755).

2.5 Thrombotic APS (TAPS)

The presence of aPl may lead to a thrombotic event anywhere in the body such as arteries, veins, central nervous system, the skin or internal organs, including spleen, liver, kidney or placenta (Sikara, Grika and Vlachoyiannopoulos, 2011, pp. 162–178) (Rovenský *et al.*, 2016, pp. 1–7). The venous system is the most affected, followed by the arterial system and the central nervous system (Rovenský *et al.*, 2016, pp. 1–7). Renal involvement is specific for SAPS (Sciascia *et al.*, 2015, pp. 478–486).

A specific pattern of occurrence has not been detected which indicates that aPl interfere in multiple ways with the haemolytic process. However, it has been shown that venous thrombosis is more likely to be followed by a venous event and arterial thrombosis by another arterial event (Rosove and Brewer, 1992, pp. 303–308).

Possible mechanisms about autoantibody-mediated thrombosis are summarized in the table below. Several mechanisms have been proposed, including an imbalance of coagulation-fibrinolysis, phospholipid proteins, which are expressed on the surface of platelets, endothelial cells and monocytes.

Table 1: adapted from: (Roubey et al., 2002, pp. 91–100) "Possible mechanisms of autoantibody-mediated thrombosis in antiphospholipid syndrome"

Inhibition of anticoagulant reactions
Inhibition of the protein C pathway
Inhibition of protein C activation
Inhibition of activated protein C
Inhibition of antithrombin activity
Displacement of annexin A5
Inhibition of β_2GPI anticoagulant activity
Cell-mediated events
On monocytes
Expression of tissue factor
Enhanced endothelial cell procoagulant activity
Expression of tissue factor
Expression of adhesion molecules
Impaired fibrinolysis
Dysregulation of eicosanoids
Decreased endothelial cell prostacyclin production
Increased platelet thromboxane A2 production
Enhanced platelet activation/aggregation

2.6 Obstetric Antiphospholipid Syndrome (OAPS)

OAPS describes a solely obstetric manifestation of APS, including complications in early and late gestation, such as recurrent foetal loss, late foetal loss, intrauterine foetal death, intrauterine growth restriction and preeclampsia. In 2006, three new pregnancy-related clinical criteria were released after the International Congress on APS (Miyakis S. *et al.,* 2006, p 295). Although OAPS is mainly diagnosed after

the occurrence of the above mentioned complications, sometimes it can be diagnosed without a prior history of thrombotic events or adverse obstetric outcome (Galarza-Maldonado *et al.*, 2013, p. 408).

Since 2010 multiple European and two center in Argentina have been able to enter their findings and observations into a registry called: *"The European registry on obstetric Antiphospholipid syndrome (EUROAPS)".* The aim of this database is to collect and release more specific results regarding the diagnosis and treatment of OAPS in order to optimize medical care for affected women around the world (Alijotas-Reig J. *et al.*, 2019, pp. 7,22).

2.7 Non-Criteria OAPS

Even though standardized classifications for OAPS have been made, multiple patients fail to meet those criteria entirely. These cases with incomplete clinical or laboratory data according to the Sydney recommendations could be classified as non-criteria OAPS (Alijotas-Reig and Ferrer-Oliveras, 2012, pp. 766–768) (Alijotas-Reig *et al.*, 2018, pp. 1–8) (Conti *et al.*, 2019, p. 3). In the clinical routine non-criteria OAPS presents a controversial issue due to the fact that in general those women do not receive treatment. However, clinical practitioners are stressing the fact that they might benefit from medical care designed for OAPS (Alijotas-Reig and Ferrer-Oliveras, 2012, pp. 766–768) (Alijotas-Reig J. *et al.*, 2018, pp. 1–8) (Conti *et al.*, 2019, p. 15). The results of a large comparative study confirmed that thesis (Alijotas-Reig J. *et al.*, 2019, p. 7).

The ”seronegative APS”, describes a special form of non-criteria APS, consisting of patients, who are lacking a sufficiently high amount of antibody titers or solely show high titers of non-criteria antibodies but with typical clinical features for APS (Salle V., 2020, pp. 1–3).

The following are the most common unconventional antibodies:

- IgA isotypes of aCL and aβ2GPI
- Antibodies directed against domain I of the aβ2GPI
- Antiphosphatidilethanolamine antibodies
- Anti -phosphatidylserine antibodies
- Anti -prothrombin antibodies
- Antiphosphadicacid antibodies
- Phosphatidylserine -prothrombin complex antibodies
- Antibodies directed against annexin A5 and A2
- Antibodies directed against phosphatidic acid

(Salle V., 2020, p. 3).

At the moment there are no standardized laboratory testing's in order to detect non-criteria antibodies. However, they may provide diagnostic aid for transient seronegativity or for patients with a strong suspicion of OAPS lacking classical antibodies (Salle V., 2020, p. 7). It is speculated, that if these non-criteria antibodies would be included in clinical routine, about 60% of patients who otherwise would be withheld treatment, would benefit from this diagnostic procedere (Liu T. *et al.*, 2020, p. 10). However, this diagnosis must be made with care and only after excluding primary and secondary causes of thrombophilic diseases (Pignatelli P. *et al.*, 2020, p. 9).

2.8 PAPS and SAPS

The definition of the primary antiphospholipid syndrome (PAPS) is the occurrence of solely APS. The secondary APS (SAPS) is linked with the coexistence of other autoimmune disorders especially SLE (Levine and Branch, 2002, p 755) (Miyakis S. *et al.,* 2006, p 295).

In a clinical study conducted over a period of two years released in 1994, the researchers included 114 patients, 58 of whom suffered from PAPS and 56 of whom had SAPS. They aimed to carry out further investigation into the difference between the two types. At the end of their study, they concluded that primary and secondary APS (apart from the fact that SAPS was more likely to include heart

valve disease, haemolytic anaemia, low c4 levels and neutropenia),- were very similar in their clinical presentation (Zuo Y. et al, 2020, pp.929-937) (Vianna J. *et al.,* 1994, p. 6).

2.9 Catastrophic Antiphospholipid Syndrome (CAPS)

CAPS is a clinical variation of APS with multi-organ involvement. Clinical symptoms presented are multi-organ failure, thrombosis of the small venous or arterial system and high titers of antiphospholipid antibodies. CAPS has a relatively high mortality rate of 50 %. Luckily, only 1% of the individuals affected by APS suffer from this rare form (Ashershon R.A. *et al.,* 2003, p. 531). About 6% of the cases of CAPS occur during pregnancy. HELLP syndrome often presents itself at the same time (Gómez-Puerta, Sanin-Blair and Galarza-Maldonado, 2009, p. 86). Nevertheless, it is very important to take every case of CAPS seriously and treat it professionally and rapidly (Asherson R.A., 1992, p. 399) (Asherson R.A. *et al.,* 2003, pp. 530-534).

2.10 Genetics

In 1966 Harvey described a family suffering from thrombotic events that also tested falsely positive for Syphilis. This was underlined by other studies that concluded that family members of individuals suffering from APS are more likely to carry different antiphospholipid antibodies. There have been studies that are prone to argue that the disease is passed on following an autosomal dominant model (Chighizola C. *et al.,* 2016, pp. 41–42).

3 Antiphospholipid antibodies

aPl are a heterogenic family of antibodies. They are reacting with phospholipid (PL) binding proteins which can be found in the blood plasma. In detail with beta 2 glycoprotein 1 (β2GPI), Prothrombin (PT) and cardiolipin (CL). (Chighizola C. *et al.*, 2016, p. 35) The antiphospholipid antibodies were first described in 1940. (Roubey R., 1996, p. 1444) The three most common antiphospholipid antibodies are the Lupus anticoagulant (LA), anticardiolipin antibody (aCL) and the anti-beta 2 Glycoprotein 1 antibody (aβ_2GPI) (Miyakis S. *et al.*, 2006b, pp. 300–302). Many studies have proposed an association between aPl and thrombosis hence the fact that the occlusion of vessels was the key event in the majority of cases (Meroni P. *et al.*, 2011, p. 332).

3.1 LA

The Lupus anticoagulant is associated with the highest risk of a thrombotic event. However, the detection of this antibody is complicated. Furthermore, the interpretation of results must always be performed with care especially during pregnancies, close to thrombotic events or during pharmaceutical treatment. Concerning the latter, further testing should be performed 2 weeks after the treatment stops (Brandt J. *et al.*, 1995, pp. 1185–1190)(Pengo V. *et al.*, 2009, pp. 1739–1740).

About 5 to 10% of the individuals with SLE are positive for LA. Patients positive for LA present with a prolonged partial thromboplastin time (aPTT), dilute Russel Viper Venom Time (dRVVT), kaolin plasma clotting time and sometimes prothrombin time (Patel and Chaney, 2007, pp. 361–364; Pengo and Five, 2016, p. 869). According to literature, LA should always be tested after a thrombotic event. However, some may argue that overtesting, e.g. using more than two tests, could lead to more false-positive results (Keeling D. *et al.*, 2012, p. 52; Clinical and Laboratory Standard Institute, 2014, pp. 3-16).

It was recommended by two guidelines that the dilute Russell Viper Venom Time (dRVVT) is the most specific, followed by the activated partial thromboplastin time

(aPTT) (Pengo V. *et al.*, 2009, pp. 1738–1739) (British Committee for Standards in Haematology, 2012, pp. 50–51).

3.2 aCL

Together with LA, the detection of aCL plays a central role in the management of APS. The test was first developed in the 1980s and has been used since then additionally with LA for diagnosis of APS (Willis, Papalardo and Harris, 2017, p. 186). The anticardiolipin antibodies have been detected using an extract of a beef heart and have been found to be directed against cardiolipin in a test originally used for diagnosis of syphilis (Pangborn M. C., 1941, p. 484). There are no exact numbers on how many people in the population have aCL. However, studies suggest that it lies anywhere between 0 and 14%. Furthermore, it has been found that elderly patients are more likely to test positive for these immunoglobulins (Miserocchi E. F., 2001, pp. 683–692). More or less 34 to 44 % of SLE patients test positive for aCL's (Sammaritano, Gharavi and Lockshin, 1990, p. 86). There is currently no standardized test for this antibody. It is suggested to carry out two tests 12 weeks apart (Keeling D. *et al.*, 2012, p. 51). Other than LA, aCL is not detected in-vitro by e.g. the thromboplastin time but with a solid phase enzyme-linked immunosorbent assay (ELISA). When using this immunoassay to detect antibodies, cardiolipin or other anionic phospholipids solid-phases are used (Harris E. *et al.*, 1983, pp. 1212–1213) and IgG, IgM, IgA and aCL antibodies can be detected in human samples (Willis, Papalardo and Harris, 2017, p. 186). Unfortunately, the test has a very low specificity. However, it has very high sensitivity and can be performed on the serum and plasma of patients (Tebo A., 2014, p. 1314) (Pierangeli S.S. et al., 2011, p. 187) (Bertolaccini M.L. et al., 2014, p. 4).

3.3 aβ2GPI

Beta-2 glycoprotein I antibody is the third most common of the antibodies. It was first detected in 1990 (Galli M. *et al.*, 1990, p. 1547; Reber G. *et al.*, 2004, p. 1860). Beta-2-Glycoprotein I can be described as an antigen or glycoprotein. It consists of five different domains. Domains 1-4 consist of 62 amino acids; the fifth domain is exceptional due to its different structure. The flexible hydrophobic loop enables it to insert itself into lipid membranes (Kristensen T. *et al.*, 1991, p. 185;

Lozier, Takahashi and Putman, 1991, p. 681; Bouma B. *et al.*, 1999, pp. 5166–5170). On the other hand, domains 1-4 are more important when focussing on the binding of antibodies (Laat B. *et al.*, 2011, p. 1916). There are different theories explaining how aβ2GPI antibodies are generated. Agents of infection, cryptic antigens on domain one or, the protein H of “streptococcus pyogenes” (Laat B. *et al.*, 2011, p. 1923) (Van Os G. *et al.*, 2011, pp. 2449–2450) and
Lipopolysaccharides (LPS) may play important roles (Ağar Ç. et al., 2011, p. 6939). There is strong evidence suggesting aβ2GPI leads to clotting within vessels or creates obstetric complications (Rohan W., 2013, p. 48). Like aCL, the aβ2GPI antibodies are detected by ELISA, however, tests are yet to be standardized (Reber G. *et al.*, 2004, p. 1861; Lakos G. *et al.*, 2012, p. 6).

3.4 Laboratory Detection

Recommendations regarding the laboratory detection and- testing of antiphospholipid antibodies were made those are outlined in the table bellow. In general, it is suggested that LA positivity is most predictive regarding thrombosis.. The clinical practitioner should take into account that results are to be interpreted differently as to whether or not the patients test positive for single or multiple aPl (Keeling T. *et al.*, 2012, pp. 47–54). (Ortel T., 2012, p. 76)*.*

It is important to notice that during medical treatment with anticoagulants or other medication that influences the blood clotting time, the testing should be postponed (Machin S. *et al.*, 1991, pp. 885–889).

Table 2: adapted from: (Arachchillage D. R. J. et al., 2015, p. 1127) (Garcia and Erkan, 2018; pp. 2010-2110) "Testing for antibodies"

Test details	Anticardiolipin antibodies	Ab2GPI antibodies	Lupus anticoagulant
Test	aCL ELISA	Ab2GPI ELISA	The LA assay
Test guidelines	Pierangeli et al 2008	None yet	Pengo et al 2009
Which antibodies are detected?	Antibodies against cardiolipin and cardiolipin bound beta 2 – glycoprotein - I	Antibodies against B2 glycoprotein I	Detects immunoglobulins that cause prolonged clotting times in vitro but are associated with thrombosis in vivo
Relevant isotypes	IgG, IgM	IgG, IgM	Not appicable
Which titres are considered positive?	Medium to high > 99th centile or >40 IgG or IgM phospholipid units	Medium to high > 99 centile of IgG or IgM phospholipid units	Not applicable
Is the test influenced by anticoagulant therapy?	No	No	Yes
Is there an overlap with other tests?	Yes with LA	Yes with LA	Yes, ab2GPI and aCL antibodies can have an anticoagulant effect but other antibodies such as antithrombin and anti-annexin V can contribute to this effect

4 The role of Antibodies

4.1 General Information

The exact mechanism how aPl lead to obstetric complications remains still unclear. It is believed that the procoagulant factors and pro-inflammatory processes are majority influences (Esteve-Valverde, Ferrer-Oliveras and Alijotas-Reig, 2016, p. 3). This exact mechanism has been revealed in 1999. ACL have been injected into murines; results showed a higher resorption rate in embryos, confirming human fetal loss; weight of the placenta and embryos within the treated mice was also observed. The mechanism behind is that aCL bind directly to the endothel, leading to an inhibition of prostacyclin, the protective factor against thrombosis. The authors supposed several explanations of an association of aPL and obstetric complications. (Blank M. *et al.*, 1991, pp. 3069–3072)**.** Further studies confirmed this early approach and thesis (Ornoy A. *et al.*, 2003, pp. 573–578) (Bakimer R. *et al.*, 1992, pp. 1558–1563).

In a paper, Espinosa G. et al illustrated multiple mechanisms in which aPl lead to a thrombus formation and obstetric complications, those can be observed in the following figure. They include the glycoprotein Protein C which, when activated works as a protective agent against exaggerated thrombosis. Protein S functions co-dependently to Protein C in a similar way, both are inhibiting the factors Va and VIIIa. APl interfere with this physiological process resulting in increased thrombus formation. Antithrombin is an agent which has an antithrombogenic effect; it can be found in the endothelium of vessels and is directed against the factors IXa and Xa as well as thrombin (Espinosa G. *et al.*, 2003, pp. 54-56). Another anticoagulant is annexin V which is crucial in the prevention of entering phospholipids into the clotting reaction, by binding it codependently with calcium (Wolgast LR. *et al.*, 2017, pp. 1412–1421). It is produced by endothelial cells and placental trophoblasts. The maintenance of a physiological blood circulation in the placenta is crucial. Different types of Annexin V can be found within the body, but there is a lack of evidence that one type is more likely to be affected by aPl than the other (Hiddink L. *et al.*, 2015). The beta-2- glycoprotein has both antiplatelet and anticoagulant as well as procoagulant abilities (Chighizola CB. *et al.*, 2016,

pp. 32–33; McDonnell T. *et al.*, 2019, p. 1,2,5). It is produced in liver cells, trophoblasts and endothelial cells. Domain one (D1) is the main target of aPl (Chighizola, Gerosa and Meroni, 2014, p. 401). The blood cells most affected by aPl are monocytes, endothelial cells and platelets (Espinosa G. *et al.*, 2003, pp. 56–59).

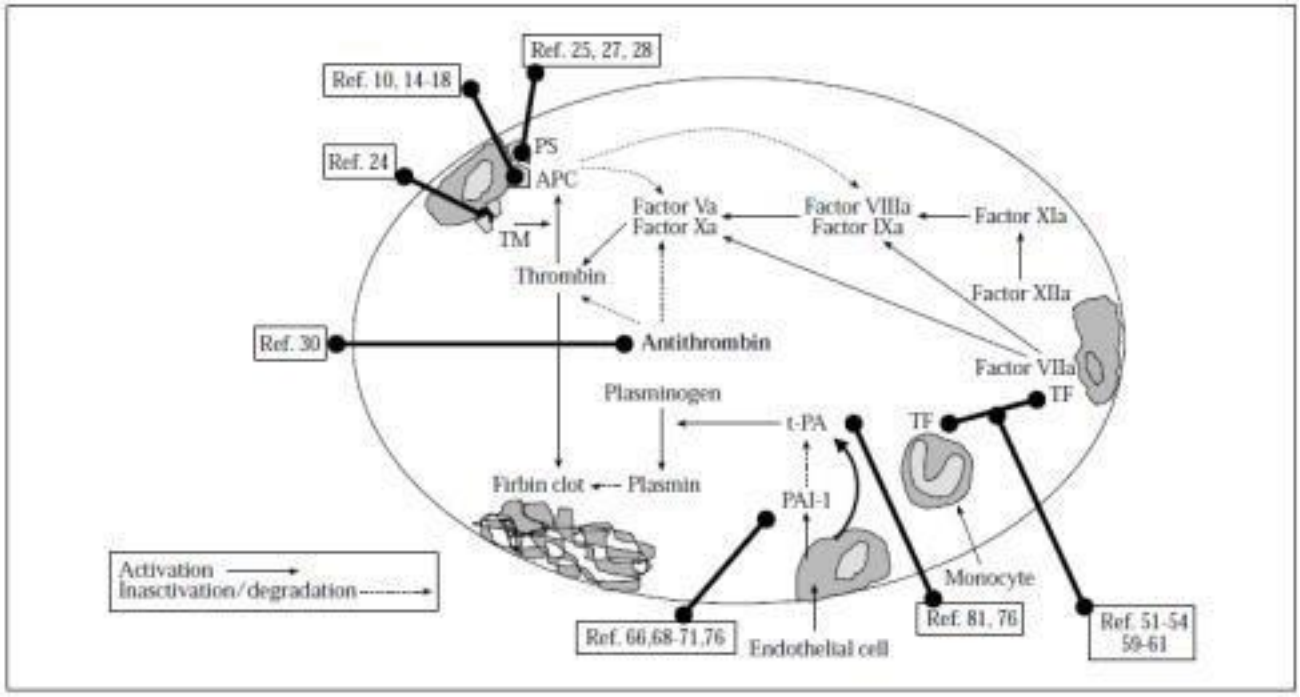

Figure 2: adapted from (Espinosa G. et al., 2003, p. 55) "Model of haemostasis pathways"

4.2 Thrombosis

One of the earliest theories is that aPl cause recurrent venous and arterial thrombosis within the placenta. That is why reduced blood flow between the mother and the foetus can be observed, leading to obstetric complications. Several authors have investigated if lower levels of prostacyclin might add to these complications (de Wolf F. *et al.*, 1982, pp. 831–833). Thromboxane and prostacyclin are both hormones that are produced by the placenta. Thromboxane has vasoconstrictive effects and prostacyclin causes the contraction of the myometrium. A study suggested a change in the amount of thromboxane production when injected with IgG of patients positive for LA. On the other hand,

prostacyclin production stayed the same. An increased amount of thromboxane then leads to spontaneous abortion (Peaceman and Rehnberg, 1993, pp. 1403–1406). Annexin V is a protein produced by the placenta which has a protecting effect against coagulation. Low levels of this protein have been found in APS patients, especially in women with a history of recurrent early pregnancy loss. Antiphospholipid antibodies may interact with the transportation of the annexin V. IgG antibodies directly decreasing the amount of annexin V on the villi surface of the placenta (Rand J. *et al.*, 1994, pp. 1570–1571).

4.3 Aβ2GPI

There are various roles aβ2GPI plays in the pathomechanism of APS during implantation and the development of the placenta (Meroni P. *et al.*, 2004, pp. 649–651). It seems that aβ2GPI adheres directly to the trophoblast, thus leading to a decreased production of humane chorion gonadotropin (hCG). A major function of hCG is to ensure adequate blood supply to the placenta and hence ensure proper nutrition. A defective implantation and placentation might lead to various obstetric complications, such as foetal loss (Di Simone N., 2001, pp. 140–149). Other studies also suggested the pathogenic role of aβ2GPI in terms of interference with the trophoblast (McIntyre J., 2003, p. 224).

4.4 ACL

It has been shown, that defective implantation, caused by aCL leads to possible foetal loss (Sthoeger, Mozes and Tartakovsky, 1993, pp. 6464–6467). A similar mechanism can be observed in the coexistence of aβ2GPI (Di Simone N., 2001, pp. 140–149). The combination of certain antibodies has the same effect (Velayuthaprabhu, Archunan and Balakrishnan 2007, pp. 272–275; Cervera R. *et al.*, 2015, pp. 3–6).

4.5 Complement System

The complement system is a series of more than 20 proteins that are activated by certain factors, e.g. microorganisms. There are four main pathways through which its activation can take place:

1. Classical pathway: C1, C2 and C4 are activated through classical antibody antigen-binding complexes.
2. Alternative pathway: C3, is activated through factors, B, D, H and I – not through classical antibodies
3. The Mannose-binding lecithin
4. The lytic pathway

(Reich N., 2008, p. 403)

Activation of the complement system might be the cause of obstetric complications (Cavazzana I. *et al.*, 2007, p. 161). When the complement system is intensely activated by aPl, ischaemia can be observed. The decreased supply of oxygen then leads to tissue injury within the placenta. Placentas of patients with APS showed an increased deposition of complement factors in comparison to placentas from normal pregnancies (Shamonki J. *et al.*, 2007, pp. 167 e1-e5). Notably, inhibition of the complement system prevents those complications (Girardi, Redecha and Salmon, 2004a, p. 1222). It has also been shown that the inhibition of C5 and C5a leads to a decreased accumulation of C3 and neutrophils and therefore fewer foetal losses and less tissue damage due to the oxygenic burst (Girardi G. *et al.*, 2003, pp. 1644–1654). The component C3 of the complement system is believed to play an important role. In an experiment, it was shown that mice injected with pathogenic antibodies showed a higher rate of resorption of embryos which is the human equivalent of early foetal loss. They also showed that the infusion of IgG that blocked the complement system prevented those obstetric complications. Those results showed further proof of the importance of the complement system and the evolution of complications of obstetric APS (Holers M. *et al.*, 2002, pp. 213–218). Apart from C3 the Complement components C5 and C5a also play a crucial role. Mice with increased C5 levels subsequently showed higher C3 levels. Due to the increased existence of complement components, more obstetric complications were subsequently observed (Girardi G. *et al.*, 2003, pp. 1644–1654).

4.6 Neutrophil Extracellular Trap (NET)

Neutrophils are cells that are activated through C5a and have also been determined to play a role in the pathogenesis of APS. A study carried out in the year 2007 showed interesting new results. They were investigating the tissue factor (TF), a protein, playing a central part in the mechanism that converts prothrombin into thrombin. However, they discovered that it was not the prothrombotic factor that was responsible for foetal loss, but the inflammatory component. They observed that the more TF they detected within the mice, the more complement activation and neutrophil activity followed. They concluded that aPl lead to complement activation, especially C3 and C5a. Due to increased activity of the complement system, exaggerated TF activation and expression on the neutrophils followed. The increased number of neutrophils with TF on their surface then leads to an “oxidative burst”. This is the production and release of reactive oxygen species (ROS) into the body. ROS are also connected to the genesis of obstetric complications such as foetal death (Redecha P. *et al.*, 2007, pp. 2423–2430). Other studies confirmed these results (Redecha P. *et al.*, 2008, pp. 3453–3461) (Martinuzzo M. *et al.*, 2005, pp. 2587–2588) (Willis, Gonzalez and Brasier, 2015, pp. 2–8).

4.7 Trophoblast and aPl

The trophoblast which forms the part of the blastocyst that is later used for nourishing the embryo is negatively affected by aPl. The implantation of the trophoblast is regulated by a complex mechanism. In general, the trophoblast consists of an inner layer, cytotrophoblast, and an outer layer, syncytiotrophoblast. Important cofactors for a physiological invasion of the trophoblast are integrins and cadherins (Aplin, Jones and Harris, 2009, pp. 299–303). Patients with aPl show a significantly lower amount of those molecules leading to an incorrect implantation of the trophoblast (Di Simone N. *et al.*, 2002, pp. 808–811). Another pathway through which antibodies interfere with the trophoblast, is inflammation. Activated by Toll-Like Receptors 4 (TLR) the TLR-4/MyD88 pathway activates the immune system. It is suggested that through this process cell apoptosis is activated and decreased vitality of the trophoblast can be observed (Mulla M. *et al.*, 2009, pp. 101–109). Apart from the implantation, the hormone production of the trophoblast

is alternated by aPl. Two crucial hormones produced by the trophoblast are the human chorionic gonadotropin (hCG) and human placental lactogen (hPL). Patients positive for APS show a decreased secretion of those hormones (Katsuragawa H. *et al.*, 1997, pp. 50–56).

4.8 Endometrium

The innermost layer of the uterus is also a target for aPl. Insufficient angiogenesis within the endometrium leads to a thin layer that subsequently can be the cause of defective placentation (Di Simone N. *et al.*, 2010, pp. 214–218). Another part of the placenta where aPl interfere with the formation of vessels is the decidua, the part of the placenta facing the mother. In murine models, it was shown that a synthetic peptide called TIFI has the ability to reverse this mechanism and restore vascular development within the placenta. They claim that after further research this could present a possible future treatment option for OAPS (Di Simone N. *et al.*, 2010, pp. 214–215, 2013, pp. 299–300).

4.9 Infertility and aPl

Primary infertility is defined by the absence of gestations after 12 months of regular unprotected intercourse (Chighizola, Raimondo and Meroni, 2017, pp. 135–146).

Multiple studies have shown that there might be a connection between the presence of aPl and infertility.

Several hypotheses have been made including that aPl interfere with the oocyte causing pathological development. Another possible mechanism is that aPL are directed against the process of decidualization preventing a physiological gestation. In vivo models with mice that have been injected with IgG aPl show decidual necrosis. Furthermore, an increased deposition of C3 and C5 within the decidua, which indicates an inflammatory process. The reduced angiogenesis within the endometrium also interferes with the proper implantation and further development of the embryo. It is suggested that exaggerated complement activation and the NET are leading to infertility through an oxidative burst and

increased inflammation (Chighizola, Raimondo and Meroni, 2017, pp. 135–146) (Chighizola and De Jesus, 2014, pp. 1232-1238) (Deroux *et al.*, 2017, pp. 78-86) (El Hasbani, Khamashta and Uthman, 2020, pp. 105-117).

It must be stated that some studies have not been limited to detect classical antibodies but also diagnosed infertility within the presence of non-criteria antibodies in women with unexplainable infertility. This might lead to confusing and inconsistent results. A homogenized laboratory testing should be provided in order to result in bias-free conclusion (Deroux *et al.*, 2017, pp. 78-86).

Nowadays, the fertility or the potency of reproduction of a woman can be investigated trough the quantity and quality of remaining oocytes. Those are observed through sonography or by testing the levels of the antimullerian hormone. There is a direct connection between the level of antimullerian hormone and the number of remaining oocytes. It has been shown that women affected by APS show significant lower numbers of remaining oocytes. It is stated that presence of aPl lead to quicker ovarian failure than in healthy control groups. However, the mechanism behind it is not yet fully understood (Chighizola, Raimondo and Meroni, 2017, pp. 135-146) (Rodrigues, Soligo and Pannain, 2019, pp. 621-627).
There is current not enough evidence proofing the connection between infertility and aPl; testing for aPl in patients with infertility is therefore not yet recommended. (El Hasbani, Khamashta and Uthman, 2020, pp. 105-117).

The following figure illustrates the above-mentioned mechanisms in which aPl interfere with the physiological blood circulation within the placenta and lead to occlusion of vessels, inflammation, modified cell activity, false or missing development of vessels and adverse function of the complement system.

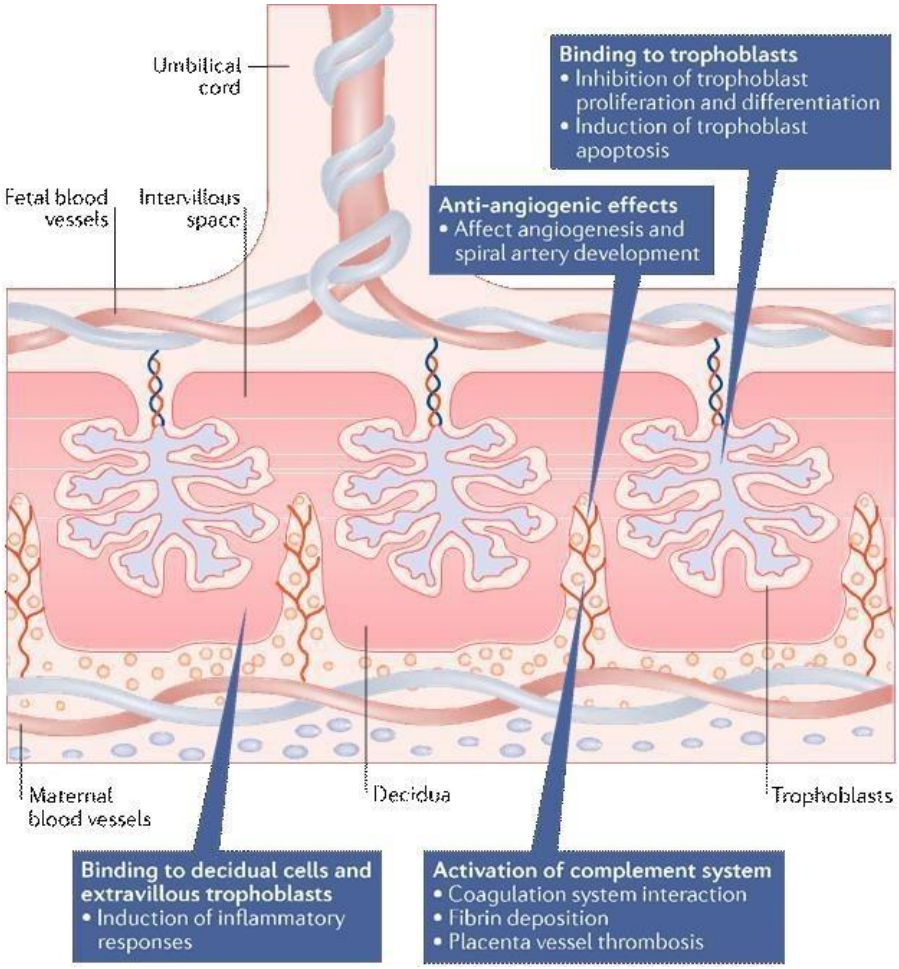

Figure 3: adapted from (Meroni P. et al.*, 2018, pp. 433–440) "Possible Pathomechanisms of aPl within the Placenta"*

5 Complications

5.1 Obstetric Manifestation

Both in the Sapporo and Sydney criteria, the obstetric complications have been listed as crucial when diagnosing APS. Recurrent early pregnancy loss (REPL), intrauterine foetal death (IUFD), severe pre-eclampsia (PE), HELLP Syndrome, placental insufficiency (PI) and intrauterine growth restriction (IUGR) are among the most common (Miyakis S. *et al.*, 2006b, p. 297). Mortality and morbidity of the mother and unborn are increased by complications. This is why the diagnosis and treatment of those is essential (Andreoli L. *et al.*, 2012a, p. 199). However, there has been a great improvement in the management of pregnant women with APS, so more women are able to carry until their estimated delivery date (Andreoli L. *et al.*, 2012b, pp. 205–206). Recurrent early foetal loss is defined as three or more consecutive miscarriages before 10 weeks of gestation; late foetal loss/intrauterine fetal death (IUFD) is defined as foetal death after 10 weeks of gestation; Intrauterine growth restriction (IUGR) is defined as < 5th percentile of gestational age. Pre-eclampsia is defined by hypertension and proteinuria after twenty weeks of gestation (Lain and Roberts, 2002, p. 3183).

Table 3: adapted from (Miyakis S. et al., 2006, p. 297) "Classification Criteria for Definite Obstetric Antiphospholipid Syndrome"

Clinical Criteria for Obstetric APS
One or more unexplained deaths of a morphologically normal foetus at or beyond the 10th week of gestation, with normal foetal morphology documented by ultrasound or by direct examination of the foetus
One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe preeclampsia defined according to standard definitions or (ii) recognized features of placental insufficiency
Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded

5.2 Recurrent early pregnancy loss (REPL)

An early pregnancy loss is the termination of gestation before 10 weeks. Among the general population, REPL can be observed in two to five percent. 10 – 20 % of the pregnancy losses can be related to aPl positivity (Wilcox A. *et al.*, 1988, pp. 192–194). Before concluding antibodies are the trigger factors, other factors, such as genetical, anatomical or hormonal aberrations, should be excluded, as they are likewise leading causes of fetal loss. (Miyakis S. *et al.*, 2006b, p. 297; Tincani A. *et al.*, 2017, p. 108). Interestingely, even under prophylactic medical treatment, pregnancy loss still occurs frequently in about 16.5 % of the cases (Tincani A. *et al.*, 2017, p. 111). However it must be stressed that so far there has not been any

clear evidence of aPl causing REPL, studies are showing controversial findings. The main difficulties were different definitions of the term early pregnancy loss and small study and control groups (Wong, Porter and De Jesús, 2014, pp. 1226–1228) (Pelusa H. *et al.*, 2017, pp. 9–10)..

5.3 Intrauterine Foetal death (IUFD)

Intrauterine foetal death is defined as foetal death after 10 weeks of gestation or a foetal weight > 500 g. Foetal death varies depending on the geographical location. In the US it can be found in about 6.4% of pregnancies (Silver R., 2007, p. 154). Other papers suggested about 2% within the general population (Goldstein S., 1994, p. 295). Patients with APS are at an increased risk with 16.9%. Receiving prophylactical treatment, this can be lowered down to 5% (Cervera R. *et al.*, 2015, p. 3). Some studies have been able to demonstrate a connection between the coexistence of aPl and FD (Herrera, Heuser and Branch, 2017, p. 2). A case report of a woman in her first gestation suffering from foetal loss, later diagnosed with APS has been presented. Receiving adequate treatment she was able to have two pregnancies without complications (Fumadó L. *et al.*, 2005, pp. 93–94). Other studies strengthened the theory that FD is often a sign for medical professionals to look further into the possibility of APS causing complications (Belhocine M. *et al.*, 2018, pp. 5–6).

5.4 Intrauterine Growth Restriction (IUGR) and Placenta Insufficiency (PI)

The placenta as a central organ of pregnancy, serves a variety of functions, such as the exchange of the respiratory gases. Hypoxemia, is the leading cause of intrauterine growth restricion. IUGR is defined as an insufficient intrauterine growth of the fetus where the abdominal circumference is found to be below the 4th percentile. Among the general population, about 3-10% experience IUGR, within APS patients it increases up to 30% (Tincani A.*et al.*, 2017, pp. 113–114). Foetuses suffering from hypoxemia are at higher risk of preterm delivery and perinatal death (Gagnon R., 2003, p. 99).

If the placenta works inadequately, every organ of the foetus is affected. Especially the development of the brain and cognitive function are impaired. This causes learning disabilities and lack of concentration later in life. Furthermore, the heart and lungs and the central organs of the cardiovascular system, are being damaged, causing a higher rate of cases of respiratory distress syndrome (RDS). In adulthood these may be causing arterial hypertension, metabolic syndrome and diabetes. The bowels are affected, leading to more incidences of necrotising enterocolitis (Gagnon R., 2003, pp. 101–104)(Audette and Kingdom, 2018, pp. 1–7).

5.5 Pre-eclampsia

Preeclampsia is a disorder affecting pregnant women, clinically diagnosed after week twenty of gestation by hypertension and proteinura.

Blood pressure levels above 140/90 mmHG or 160/110 mmHG and levels of protein in the urine of more than 300 mg in 24 hours are the main biological markers for the diagnosis. Around five percent of all pregnancies are affected by this disease with an increasing incidence after 35 weeks of gestation (Roberts J.L. et al, 2013, pp. 1122-1131) (Turner J., 2002, p. 328).

Early severe preeclampsia among the general population lies at about 2 to 8%. Within APS patients the incidence is higher, -about 20-50% of pregnant women are affected (Lockshin, 2013, pp. 209–216). Eclampsia is the onset of seizures in a woman with pre-eclampsia (Jaatinen and Ekholm, 2016, p. 1) (Turner J., 2002, p. 329). Due to the elevated blood pressure all organs are suffering from hypoperfusion, resulting in hypoxia and growth restriction (Roccella E. J., 2000, p. 2).

Clinically the distincition between early onset PE prior to week 34 of gestation and late onset PE after the 34th week of gestation can be made. Generelly pre-eclampsia is a progressive process, in some cases initial mild pre-eclampsia can develop into severe within days or even hours, called fulminant pre-eclampsia, a serious form of this syndrome. In general the blood pressure of pregnant women should be observed closely due to the high morbidity and mortality that follows

high blood pressure (Roberts J.L. et al, 2013, pp. 1122-1131). Furthermore, severe and non-severe pre-eclampsia can be distinguished. Non-severe pre-eclampsia occurs if a woman in her second or third trimester is suffering from hypertension (140-160mmHg systolic, 90-110mmHg diastolic) and elevated protein levels in her urine. In comparison severe pre-eclampsia clinically includes hypertension above 160/110 mmHg, proteinuria, decreased urine output, cephalea, abdominal pain, decreased thrombocyte level and pulmonary oedema due to water retention. Furthermore, obstetric complications such as oligohydramnios, placental abruption and decreased foetal growth (Marchetti, de Moerloose and Gris, 2016, p. 676) (Martinez-Fierro M.L. *et al.*, 2018, pp. 1–2).

The National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy listed some crucial factors leading to a faster diagnosis of pre-eclampsia (Roccella E.J., 2000, p. 3). In 2013 the task force revised existing recommendations for PE and eliminated proteinuria as one of the severe features of pre-eclampsia (Roberts J.L. et al, 2013, pp. 1122-1131).

Inlcuded have been the following severe features of pre-eclampsia:

- BP over 160/110mmHg
- Elevated serum creatinine
- Thrombocytopenia
- Elevated liver parameters
- Clinical signs such as cephalea, defect of vision or abdominal pain

The association between titers of aPl and preeclampsia has been studied by multiple teams over the past years. In the past, controversial results have been presented (Schjetlein R. *et al.*, 1998, p. 87; Dreyfus M., 2001, pp. 32–33; Lee R., *et al.*, 2003, pp. 297–298). However, more recent studies suggest a correlation. An interesting fact is that multiple positive antibody titers show a more difficult course of events than single positive variations. Moreover, lifestyle factors such as BMI, cigarette smoking or alcohol comsumption influence the negative progression of preeclampsia (Yamada H. *et al.*, 2009, p. 190). Another study suggested that antibodies are more likely to be linked with cases of severe than non severe

preeclampsia. A connection was found especially with women that are postive for aβ2GPI (Marchetti, de Moerloose and Gris, 2016, pp. 680–681) rather than LA or aCL. Other studies pointed out that the titres of antibodies fluctuate, levels rise during pregnancy and decrease post partum (Al-Balushi M. *et al.*, 2014, pp. 478–485).

5.6 HELLP Syndrome

The HELLP Syndrome is defined as a severe form of preeclampsia that occurs in the third trimester of pregnancy. It is clinically characterized by abdominal pain, usually in the upper right quadrant, nausea and sometimes neurological symptoms such as headache, sensibility to light and eye flickering. The name is an abbreviation for H; Haemolysis, EL; elevated liver enzymes and LP; low platelets. These are the laboratory features that can be observed. Complications include abruptio placentae, liver rupture, intracranial bleeding or acute kidney failure. In some cases, if high blood pressure or proteinuria is not present, the diagnosis can be difficult (Shah, Daigavane and Dsouza, 2016, p. 49). Several case reports can be found where the HELLP syndrome is first diagnosed in women that are later diagnosed with APS (Le Thi Thuong D. *et al.*, 2005, p. 275,277; Appenzeller S. *et al.*, 2011, pp. 116–117).

5.7 Thrombosis

A vessel consists of three layers: the intima, the media and the adventitia. The intima faces the lumen of the arteria or vein. Haemostasis is a physiological process that occurs when a vessel is damaged. Primary and secondary haemostasis can be distinguished. Vasoconstriction and the activation of platelets are the immediate response to damage. Secondarily a variety of coagulation factors are activated. Temporarily a small thrombus is formed close to the injured part of the vessel wall. When a pathological thrombus forms and occludes a vessel, one is speaking of thrombosis. This may occur in any part of the body. In the endothelium three thrombo-regulators can be found. Nitric oxide, prostacyclin and ectonucleotides CD39. They are important in the prevention of pathological thrombus development. Following an event that leads to the exposure of parts of

the vessel, such as collagen or tissue factor (TF), that are usually not in contact with flowing blood the coagulation process starts. Collagen found in the subendothelial matrix will when exposed to flowing blood activate platelets and support the accumulation of those blood cells. Tissue factors located in the medial (smooth muscle) and adventitial layers of the vessel wall on the other hand initiate the generation of thrombin which is a factor that again leads to even further activation of platelets and converts fibrinogen to fibrin (Furie and Furie, 2008, pp. 942–946).

5.8 Thrombotic Manifestations

In a large study carried out around the year 2000, 1000 patients were included to further investigate the clinical and immunological features of APS. Within 1580 pregnancies the most common complications have been early and late foetal loss which have occurred in approximately half of the pregnancies. Among the 590 women pre-eclampsia occurred in 9,5%, eclampsia in 4,4% and abruptio placentae in 2%. The most common thrombotic event has been the deep vein thrombosis with 39%. Osteoarticular manifestations are mostly represented by arthralgia with 38%. Low platelet count has been observed in 29% of cases. Livedo reticularis has been identified as the most common cutaneous manifestation with 24%. Cerebrovascular manifestations showed that 20% of the cohorts suffered from migraine, followed by 19% strokes and 11% transient ischemic attack. Occlusion of a pulmonary vessel has occurred in 14%. In 11% of the 1000 cohorts impaired function of the heart valves has been observed as the most common cardiac manifestation (Cervera R. *et al.*, 2002, p. 1023).

5.9 Vascular Manifestation

There is evidence that APS can lead to both thrombophilia and vasculopathy. There are various techniques that can be used to examine the functionality of vessels. For example, the ankle brachial index (ABI), increased carotid artery intima media thickness or histopathological findings. Furthermore a reduced endothelial -dependent flow mediated dilation (Christodoulou and Sangle, 2007, pp. 908–909; Siddique S. et al., 2017, pp. 2,7-8). The deep vein thrombosis,

mostly found in the lower extremities, is the most common clinical feature within APS patients (Cervera R. *et al.*, 2002, p. 1023). Furthermore, the heart, lung, bowels and kidneys are most likely to be affected. Damage of the valves, partial or complete occlusion of vessels or malfunction of the ventricles can be observed within the heart. The lung is affected by high blood pressure. Kidney and bowels are broadly affected by stenosis of supplying arteries (Christodoulou and Sangle, 2007, p. 908).

5.10 Obstetric APS and Thrombosis

Often APS is diagnosed due to recurrent foetal loss. This raises the question if APS existed before or only occurred due to the pregnancy. Furthermore, the question of prophylactic thrombotic postpartum treatment has been raised. In a study carried out in 2017 it was found that in a 10 year follow up there was a 10.4% chance of women developing thrombotic events (Drozdinsky G. *et al.*, 2017, p. 3). Other studies confirmed that theory, (Quenby, Farquharson, Dawood and Hughes, 2005, p. 1730; Teo K. *et al.*, 2006, pp. 1000–1001) some suggesting post partum treatment with Heparin (Erkan D. *et al.*, 2001, pp. 1466–1467).

5.11 Pregnancy and SLE

Multiple specialists observed that systemic inflammatory diseases such as SLE or APS tend to appear or worsen during the reproductive peak of a woman. Furthermore, observations showed that women which have suffered from obstetric complications in the past had successful following pregnancy. This stresses the fact that pre-conceptional counselling, planning of a pregnancy and strict as well as continuous assessment throughout the gestation are key factors ensuring a successful outcome (Vagelli, Tani and Mosca, 2017, pp. 115–121) (Specker C., 2011, pp. 397–414) (Schreiber K., 2016, pp. 343–345).

There is no evidence that either SLE or APS lead to a higher number of infertile women. However, most affected patients may be put under stress by active symptoms and the concern of possible interference of the disease with a pregnancy. This can create the appearance of a seemingly higher infertility rate. To prevent this negative effect of decreased birth rate, education regarding the effective treatment possibilities must be performed (Fischer-Betz and Specker,

2017, pp. 397–414). Apart from the fact that an autoimmune disease can affect a possible pregnancy, the pregnant status of a woman itself can affect the clinical picture of those diseases. Within SLE the so-called flare, which is an increased activity of the disease in one or multiple organ systems, leading to worsening or even the new appearance of clinical signs and symptoms, can appear more frequently. Mostly the kidneys and the haematopoietic organs as well as the blood are affected (Petri M., 2007, pp. 227–235) (Guettrot-Imbert G. *et al.*, 2015, pp. 173–181).

The differentiation between physiological sign and symptoms accompanying a pregnancy and a flare is challenging even for practitioners with an extensive knowledge and clinical experience (Ruiz-Irastorza and Khamashta, 2004, pp. 679–682). However, a differentiation must be made in order to apply suiting treatment either for a flaring woman or to prevent possible adverse effects of SLE on the unborn baby. The unexpected loss of a pregnancy, IUGR, pre-eclampsia or a delivery prior to the estimated date of birth are among the most common obstetric manifestations caused by the autoimmune disease (Fischer-Betz and Specker, 2017, pp. 397–414) (Schreiber K., 2016, pp. 343–345). The combination of SLE and the existence of aPl are leading to even higher numbers in obstetric complications in comparison to women suffering solely from SLE or OAPS (Petri M., 2007, pp. 227–235).

6 Diagnosis

6.1 General Information

It must be mentioned that the standardized clinical and laboratory criteria were not designed for routine clinical diagnosis, but as inclusion criteria for clinical studies (Gardiner C. *et al.*, 2013, p. 1). That is the reason why diagnosis within the clinical routine is sometimes still challenging (Miyakis S. *et al.*, 2006, p. 297). Additional clinical information has been given and must be taken into account when diagnosing APS in order to prevent misdiagnosis. Regarding vascular thrombosis, the risk factors are an age above 65 years within males and 55 years within females, high blood pressure, diabetes mellitus, dyslipidaemia, abuse of nicotine, family members with premature cardiovascular diseases, a body mass index (BMI) over 30kg/m², a glomerular filtration rate (GFR) of less than 60 mL/min or microalbuminuria. Oral contraception, nephrotic syndrome, malignancy, immobilization, surgery and inherited thrombophilias must be considered additionaly. Patients with any of those risk factors stated above should be assessed with proper care to prevent the misdiagnosis of APS. (Miyakis S. *et al.*, 2006, p. 297).

Concerning laboratory criteria it is crucial that patients are categorized in subgroups differing if multiple or single antibodies are detected (Miyakis S. *et al.*, 2006, p. 297).

Non-criteria clinical and laboratory manifestations might complicate the diagnosis. A diagnosis of non-criteria obstetric APS is considered if the patient has: a) a combination of non-criteria clinical manifestations with international consensus laboratory criteria; or b) international consensus clinical criteria with a non-criteria laboratory manifestation (Gardiner C. *et al.*, 2013, p. 1; Arachchillage D. *et al.*, 2015, p. 14).

Sometimes women do not fulfill the diagnostic criteria of APS or OAPS. However they would still benefit from antithrombotic treatment (Alijotas-Reig and Ferrer-Oliveras, 2012, p. 767) (Mekinian A. *et al.*, 2012, p. 225).

It must be stressed that the rapid diagnosis of APS within the pregnant population or prior to a planned pregnancy is important due to the fact that rates of depression, general anxiety and overall health are affected areas, especially by obstetric complications such as recurrent foetal loss (Craig, Tata and Regan, 2002, p. 160). It is stated that affected women visiting hospitals who are experiencing foetal loss or (recurrent) miscarriages are handed out forms to evaluate their psychological health. Women showing high scores are later interviewed and if necessary given the opportunity to receive cognitive behaviour therapy. This special kind of psychotherapy can be applied through a specialist or at home with the use of the internet. Both forms of cognitive behaviour training are showing significant reduction of anxiety and depression in women experiencing obsteric complications which leads to an improved quality of life. It is stressed that mental health is as important as their physical health. The different options for receiving help regarding the psychological health should be explained and proposed at all time, not only during times of mental stress or after an obvious impairment of quality of life. (Craig, Tata and Regan, 2002, p. 160) (Tavoli *et al.*, 2018, pp. 1–5)

6.2 Who should be tested?

In general there are a lot of scenarios in which patients are tested for APS.

1. One of those are incidental findings. Keeling et al recommended that even if positive aPl are accidentally found no steps should be taken if the patient is asymptomatic (Keeling D. *et al.*, 2012, p. 52).

2. Another scenario is that you have a patient in your care with thrombosis. In that case if you are treating a younger patient (under 50 years of age) with arterial thrombosis, recurrent thrombosis, a combination of venous and arterial thrombosis or unprovoked venous thrombosis further steps into

investigating and testing should be taken (Garcia and Erkan, 2018b, pp. 2014–2015). Due to the fact that prophylactic therapy is not indicated in patients with unprovoked thrombosis or due to transient risk factors no further investigation for APS should be conducted within these patients (Keeling D. *et al.*, 2012, p. 52). If you are dealing with patients that suffered a thrombotic event in an atypical part of the body screening for aPl is recommended (Garcia and Erkan, 2018b, pp. 2014–2015).

3. Within the group of SLE patients it is recommended that an APS diagnostic should be initiated in the case that the patient would be in a situation with an elevated risk of a thrombotic event. These include pregnancy, surgical operations, transplantation or oestrogen treatments. Furthermore, if SLE patients suffer from a vascular, neurological or obstetric event (Garcia and Erkan, 2018b, p. 2014).

4. The fourth scenario includes patients with ischaemic stroke. It is recommended that only young patients under the age of 50 years should be tested for aPl (Keeling D. *et al.*, 2012, pp. 52–53) (Giannakopoulos B. *et al.*, 2009, p. 990).

Within the pregnant patient group testing should be carried out if there is a prolonged aPTT in routine laboratory testing. Furthermore, if recurrent early pregnancy loss (3 or more before 10 weeks of gestation) or provoked venous thrombosis can be observed. Patients suffering from autoimmune disorders such as SLE, autoimmune thrombocytopenia, autoimmune haemolytic anaemia or rheumatoid arthritis should also be tested. It must be stressed that the diagnosis without any evidence should be avoided due to the risk of false positive test results that lead to unnecessary treatment (Miyakis S. *et al.*, 2006b, pp. 295–306) (Keeling M. *et al.*, 2012, pp. 47–58). It must be stated that an investigation should only be carried out during pregnancies as the treatment might affect the results (Keeling M. *et al.*, 2012, pp. 47–58).

6.3 Follow Up of Children

In general, the antiphospholipid syndrome is diagnosed around the age of 30, early onset forms of this disease even affecting new-borns have been observed. This has raised the question if women positive for aPl are able to transfer those antibodies to their infants. Another hypothesis is that the elevated levels of aPl occur due to the prematurity of the new-born. Some even suggested a genetical form of APS leading to complications as early as in the perinatal time and not as more commonly observed later in life (Tincani *et al.*, 2009, pp. 70-76) (Mocková *et al.*, 2012, pp. 793-795).

In around 30 % newborns of women with APS or OAPS, are tested positive for said antibodies. However, those numbers might be altered due to the fact that nowadays the majority of mothers are receiving prophylactic treatment which is able to bind circulating antibodies and preventing the transfer between mother and foetus (Tincani *et al.*, 2009, pp.70-76). Noteworthy is that after birth, titers of antibodies have been detected in blood samples of newborns, but disappeared within 12 months to 2.5 years (Mekinian A. *et al.*, 2013, pp. 218–221) (Berkun *et al.*, 2014, pp. 986-993).

It has been observed that the presence of aPl can lead to perinatal arterial ischemic stroke (PAS) or cerebral sinus vein thrombosis (CSVT). Those are infants fulfilling the clinical and laboratory criteria for APS. New-borns have been born to healthy mothers or mothers with OAPS. At the end of the follow up period 75% showed reduced neurological development. However, no other manifestations of APS have been observed over this period. It can be said that elevated levels of aPl are not rare among children, but thrombosis is. This stresses the fact that there has to be an additional trigger leading from solely elevated serum levels to a thrombotic event in the perinatal period (Berkun *et al.*, 2014, pp. 986-993) (Merlin, 2011) (Alshekaili, Reynolds and Cook, 2010, pp. 1565-1568). In another large study conducted in 2015 no evidence was found that children from mothers with APS are more likely to suffer from future thrombotic events or SLE. On the other hand the neurological development was in some cases not as progessive as within the control group (Mekinian A. *et al.*, 2013, pp. 218–221).

This might be a reason for follow up of neurological development (Mocková *et al.*, 2012, pp. 793-795).

Even tough new-borns sometimes fit the international diagnostical criteria paediatricians are stressing the fact that they are not as applicable to infants. It is claimed that children are not in their childbearing ages, having different hormonal levels than grownups. Furthermore, they lack all additional risk factors leading to recurrent thrombotic events (Berkun *et al.*, 2014, pp.986-993) (Merlin, 2011) (Alshekaili, Reynolds and Cook, 2010, pp. 1565-1568).

In conclusion it can be said that during the perinatal period and even during gestation antibodies are passing from the mother to the unborn child. A transfusion leading to a manifestation of APS among newborns has not been observed so far. However, the central nervous system of the infants is affected leading to anatomical abnormalities, vessel occlusion and in later development might lead to impaired brain function as well as learning disabilities. Closer medical attention as well as neurocognitive training is therefore recommended in these children. (Tincani *et al.*, 2009, pp.70-76).

7 Treatment

7.1 OAPS Treatment

Due to the quite high rate of pregnancy complications in women with APS, adequate treatment from the beginning of the pregnancy, is important (Drakeley, Quenby and Farquharson, 1998b, p. 1976). Pregnancies are successful in only 20% to 30% of cases without adequate treatment (Chighizola C. et al., 2014, p. 1508) (Schreiber and Hunt, 2019, p. 44). That is why, pre-conceptional counselling and close medical attention throughout the gestation are crucial. Currently pregnancies of woman with APS are classified as high-risk pregnancies.
In general, it was observed that the success rate of pregnancies increased due to an improvement of management over the last decade (Cervera R. et al., 2015, pp. 4–6).

The pathophysiology of complications at early gestation, such as recurrent foetal loss differs from those of late gestation. First trimester miscarriages seem to be due a direct inhibitory effect of aPl on proliferation of trophoblast cells, whereas preeclampsia, IUGR and stillbirth are a consequence of placental dysfunction. APl play an important role by reducing proliferation and invasion of extravillous trophoblasts and triggering inflammation at the materno-fetal interface, thus leading to impaired placentation. When treating OAPS patients, those mechanisms are to be controlled. In order to reduce vascular occlusion anticoagulants and antiaggregants are being used. For the other part intravenous immunoglobulins, which are proteins that mark antibodies so the immune system of the patient can target them, and glucocorticoids are administrated. Those are discussed later in the thesis (Schreiber and Hunt, 2019, p. 42).

7.2 Medication

The basic treatment of antiphospholipid syndrome in pregnancy is low-dose-aspirin and low-molecular- weight-heparin. Heparin has a variety of actions including anticoagulant activity and inhibitory actions on vascular smooth muscle cell proliferation and migration, as well as anti-inflammatory effects (Dilley and

Nataatmadja I, 1998, pp. 247–255; Kohno *et al.*, 1998, pp. 1065–1069) (Sawhney *et al.*, 1997, pp. 611–614). Additionally, heparin has been shown to inhibit complement activation in trophoblasts and therefore might prevent pregnancy loss (Girardi, Redecha and Salmon, 2004, pp. 1222-1226).

According to literature, aspirin improves endometrial growth, placental vascularization, implantation and placentation and has vasodilatory effects by increasing prostacyclin production. Aspirin seems to have a direct effect on platelets, might improve endothelial dysfunction (Tranquilli *et al.*, 2014, pp. 97–104) and has a modest beneficial effect in reducing the rate of preterm preeclampsia (Roberge *et al.*, 2012, pp. 141–146; Villa *et al.*, 2013, pp. 64–74; Tong, Mol and Walker, 2017, pp. 95–97; Story and Nelson-Piercy, 2018, pp. 613–622). This benefit might only be achieved if low-dose aspirin was started before 16 weeks of gestation. Several studies have evaluated the potential benefit of administration at early gestation or even preconceptional of low-dose aspirin in women with APS (de Vries *et al.*, 2012, pp. 64–72; Amengual *et al.*, 2015, pp. 1135–1142; Areia *et al.*, 2016, pp. 81–86; Saccone *et al.*, 2017, pp. 1–12). However, a recent systematic review has evaluated a probable effect of aspirin initiated before 11 weeks of gestation in women at high risk for the development of preeclampsia, such as history of recurrent foetal loss, thrombophilia or APS. The authors revealed that the administration of low-dose aspirin before 11 weeks of gestation did not reduce the risk of preeclampsia, and foetal growth restriction but might reduce the risk of preterm delivery at <37 weeks of gestation (Chaemsaithong *et al.*, 2020, pp. 437–450).

At the moment, a combination of low dose aspirin (LDA) (100-150 mg/day) and low molecular weight heparin (LMWH) is used for treatment. Multiple studies showed an advantage of the combination rather than single usage (Kutteh W., 1996, pp. 1587–89; Elmahashi M. *et al.*, 2014, p. 3; Schreiber, Radin and Sciascia, 2017, p. 4). When receiving therapy, a significant improvement regarding live birth rates and thrombotic events is shown. In studies the number of viable infants almost doubled under treatment (Kutteh W., 1996, pp. 1587–89). Other studies showed similar results regarding pregnancy outcome (Bramham K. *et al.*, 2010, pp. 60–63). However, obstetric complications caused by aPl are still frequent which calls for further investigation into the pathomechanism and

management of this population (Schreiber and Hunt, 2016, p. 5) (Chighizola, Shoenfeld and Meroni, 2018, p. 2). Treatment should continue for 6- 12 weeks after birth (Antovic A. *et al.*, 2018, p. 5). Due to their complement deactivating effects Corticosteroids in a decreased dose, may be used within the first three months of pregnancy. However, they are also linked to the development of gestational diabetes as well as preterm deliveries which are limiting factors when using Prednisolone or other substances from this pharmacological class (Tsagalis G. *et al.*, 2010, pp. 215–216; Mayer-Pickel K. *et al.*, 2017, p. 9). Direct oral anticoagulants and vitamin K antagonists should not be used due to their ability to cross the placenta (Schreiber and Hunt, 2019, pp. 41–46).

In the following flow diagram, the therapeutic approach of OAPS is illustrated.

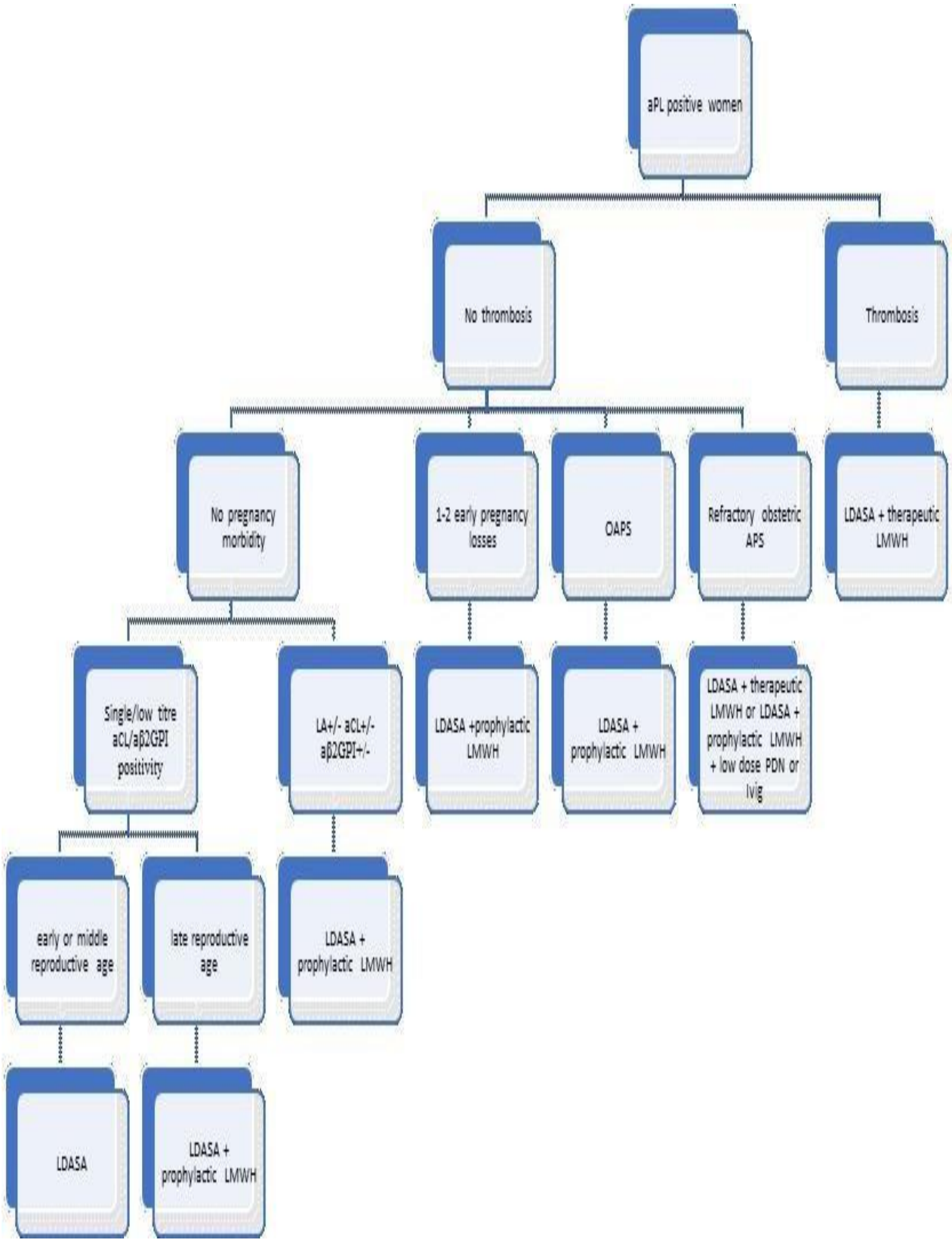

Figure 4: adapted from (Chighizola C. et al., 2014, p1513) "Flow diagram of therapeutic approach to OAPS"

7.3 Counselling

In general, there are no reasons why women diagnosed with APS or positive levels of aPl should not conceive. Coexisting diseases such as uncontrolled arterial hypertension, diabetes mellitus, nicotine and alcohol abuse are reasons for more intense counselling and monitoring due to the higher morbidity and mortality (Chighizola C. *et al.*, 2014, p. 1507). Regular follow-ups monitoring blood pressure, urine investigations especially proteinuria, blood samples emphasizing platelets and sonography of the placental vessels are important during gestation (Chighizola C. *et al.*, 2014, p. 1508).

Some reasons that may lead to advising a woman against conceiving are an active SLE, especially if alternated kidney function is involved. If existing arterial hypertension can only be adjusted inadequately, thrombus formation in a vessel supplying the central nervous system in the past medical history or the occurrence of severe pregnancy complications even though prophylactic treatment has been applied are other factors influencing the decision (Specker Ch., 2011, p. 6).

Specialists emphasize women and obstetricians to conduct a vaginal birth and not a caesarean section. The main reason being that after delivery there is a lower chance of thrombosis (Schreiber and Hunt, 2019, p. 43).

7.4 Plasmapheresis

Plasmapheresis represents a new reliable form of treatment for OAPS patients. Research suggests that monocytes are central in the immunological mechanism. An increased cellular response of Th1 (T helper cells 1) instead of Th2 (T helper cells 2) is common within pregnant women with APS. The main difference between the two is their secretion; Th1 mainly produce interferon-y (IFN-Y) and tumour necrosis factor-α (TNF-α) whereas Th2 secrete interleukins 4,5,10 and 13. However, the shift from a mainly Th1 triggered immunological response to Th2 is crucial for a successful pregnancy. Furthermore, a downregulation of CCL2 and upregulation of CXCL10 are present within patients. These cytokines are produced during the inflammatory response by monocytes. Following plasmapheresis an

upregulation of CCL2 but no changes regarding CXCL10 are observed regarding CXCL10 (Martirosyan A. *et al.*, 2016, p. 1193).

This alternative treatment for OAPS has shown positive outcome effects in many studies. Especially the combination of dialysis and intravenous immunoglobulins shows a significantly lower rate of complications during pregnancy (Mayer-Pickel K. *et al.*, 2017, pp. 11–12). Another study that focused on patients positive for all three types of antibodies and multiple complications in prior pregnancies also presented as positive outcome (Ruffatti A. *et al.*, 2016, pp. 14–19). In 2007 a small group of patients received plasmapheresis combined with low dose prednisolone (10mg daily). The study showed an increased live birth rate (100%) and decreased a number of obstetric complications (El-Haieg, Zanati and El-Foual, 2007, pp. 239–240).

7.5 Intravenous Immunoglobulins

Immunoglobulins are proteins retrieved from human serum which are broadly used in the treatment of autoimmune disease. There are multiple mechanisms in which they contribute to the treatment of those diseases. Among APS and OAPS it has been shown that they decrease the production of aPl as well as inhibit their activity (Tenti S. *et al.*, 2016, pp. 8–9).

For example, it has been proven that the administration of intravenous immunoglobulins inhibits the binding of aPl to the trophoblast, which is central in the development of obstetric complications (Bakimer R. *et al.*, 1993, p. 101).

At the moment, they are only used as a second line therapy within pregnant women who do not respond to standardized treatments. Multiple studies have shown that besides having a very low rate of adverse effects they have been effective, resulting in elevated life birth rates (Galarza-Maldonado C. *et al.*, 2013, pp. 408–409) (Watanabe N. *et al.*, 2014, p. 299) (Ruffatti A. *et al.*, 2018, p. 7). However, other authors claim that apart from the fact that immunoglobulins are expensive and often unavailable, clear evidence for the effectiveness of immunoglobulins is still missing and further investigation has to be taken into this

topic in order to justify this high cost therapeutic approach (Ruffatti A. *et al.*, 2017, p. 6).

7.6 Treatment Perspectives

At this time approximately 70 % of the pregnancies can be adequately managed by treating or even preventing pregnancy complications. However, the remaining 30% of women are still suffering from the traumatic experiences followed by obstetric complications (Schreiber and Hunt, 2019, p. 44).

7.7 Hydroxychloroquine and Statins

Hydroxychloroquine (HCQ) and statins are promising medications that may be used in the future when sufficient investigation has been made and evidence has been found (De Jesus G. *et al.*, 2014, p. 89) (Mekinian *et al.*, 2015, pp. 498–502) (Sciascia *et al.*, 2016, pp. 273.e1-273.e8).

Hydroxychloroquine primarily used as an anti-malaria treatment has found its way into other therapeutic schemes such as for the treatment of autoimmune diseases like SLE, rheumatoid arthritis and APS. It is known to have an anti-inflammatory effect due to its immunosuppressive properties which makes it suitable to treat the former mentioned diseases. The mechanism in which way hydroxychloroquine prevents the formation of a thrombus among APS patients has been investigated in multiple studies (Belizna C., 2015, pp. 358–362). Still, the exact mechanism remains unclear. Antithrombogenic effects are probably due to decreased platelet aggregation caused by reduced alpha-granule release and adenosine diphosphate activity. Furthermore, inactivation of phospholipase A2, inhibition of calcium which is bound to the membrane of thrombocytes or throughout decreased release of arachidonic acid by thrombocytes. The administration of hydroxychloroquine and chloroquine have shown to decrease platelet aggregation (Achuthan S. *et al.*, 2015, pp. 174–180) (Janč inová, Nosál and Petriková, 1994, pp. 495–504) (Bertrand E. *et al.*, 1990, pp. 143–146). Some studies have shown a reduced haematocrit after the administration of HCQ opening the question if the drug additionally effects erythrocytes (Bird, Harkness and Wright, 1981, pp. 36–39) (Ernst, Rose and Lee, 1984, pp. 48–52). In a study conducted in 2008 scientist

were able to show that the administration of HCQ can prevent the binding of aPl and phospholipid bilayers which is one of the central pathomechanism in which the aPl lead to thrombosis followed by obstetric complications (Rand JH: *et al.*, 2008, pp. 1687–1695).

Due to the most probable inflammatory component of the pathophysiology glucocorticoids are being investigated. Some studies advise against the use of steroids others suggest that a low dose prednisolone therapy may elevate live birth rates and decrease pregnancy complications (Bramham K. *et al.*, 2011, p. 8).

7.8 After birth treatment

Regardless of the fact if APS has been diagnosed before, during or after a pregnancy affected women should receive close medical attention after delivery (Schreiber and Hunt, 2019, p. 44). Specialists are advised to follow the current treatment trends that were summarized after the 15th international congress in 2016 in the northern part of Cyprus (Andrade D. *et al.*, 2017, pp. 317–338).

8 Discussion

The results of this literature research indicate a strong connection between the existence of aPl and pregnancy complications.

To start with a meanwhile well-known fact is that approximately 80 percent of all APS patients are women. About five percent of unsuccessful pregnancies are due to aPl. Therefore, the existence of antibodies must be considered as important. The main plasmatic proteins that bind to antiphospholipid antibodies are beta-2-glycoprotein, prothrombin, protein c/s and annexin V. Most domains or binding agents as well as the roles they play in the coagulation cascade have been successfully detected.

Antiphospholipid antibodies are a heterogenic group of antibodies. LA, aCL and aβ2GPI are described extensively. LA when present alone is the most likely to lead to complications. However, the presence of multiple antibodies is even more dangerous. The laboratory detection has recently been summarized by David Garcia and Doruk Erkan in 2018. (Garcia and Erkan, 2018b, pp. 2010–2110). This facilitates the detection as well as the comparison and interpretation of results in the clinical routine.

The literature research of older as well as more recent studies showed that, although there have been some breakthroughs a variety of pathways are still assumptions and could not be proven.

Some central ideas include:

1. The anti-angiogenic effect which leads to a decreased vessel production.

2. aPl bind directly to the trophoblast which inhibits the development of it or even induces apoptosis. Scientific findings suggest the adhesion of aβ2GPI to the trophoblast leads to a decreased production of hCG, a central mediator of a successful pregnancy.

3. The binding of aPl to decidual cells leads to inflammation and thrombosis. The occlusion of vessels within the placenta leads to an improper blood flow between the mother and foetus inhibiting optimal development.

4. Thromboxane and prostacyclin, two hormones important for the maintenance of a physiological pregnancy, are found in modified serum levels within pregnant women diagnosed with APS.

5. ACL is suspected to play a role, but facts have yet to be delivered. Laboratory testing within mice has shown that high titres of ACL lead to incorrect implantation in the early stages of pregnancy. However, the exact mechanism behind it remains a mystery.

6. Recent studies suggest that an increased activation of the complement system leads to ischemia within the placenta. The components C3 and C5/5a are found in higher serum levels leading to increased TF activation leading to the activation of neutrophil granulocytes and an oxidative burst.

In comparison to the still extensive amount of questions that are open regarding the exact pathomechanism, complications resulting from the presence of aPl are described intensively in many books, papers and studies. Among the most common are recurrent early pregnancy loss, foetal death, preeclampsia, HELLP-Syndrome and intrauterine growth restriction. They have been included in the diagnostic criteria of Sydney making the diagnosis of OAPS easier and therefore the management of high-risk pregnancies resulting in increased successful outcome numbers.

The diagnosis is still challenging in some cases although diagnostical criteria have been established. Even though the treatment has a few of complications and side effects misdiagnosis should be avoided. Important factors influencing misdiagnosis are high blood pressure, diabetes mellitus, dyslipidaemia, abuse of nicotine, family members with premature cardiovascular diseases, a body mass index (BMI) >

30kg/m², a glomerular fraction rate (GFR) of less than 60 mL/min -1 or microalbuminuria. Oral contraception, nephrotic syndrome, malignancy, immobilization, surgery and inherited thrombophilia must be considered additionally.

In the final chapter of the thesis current treatment trends as well as treatment perspectives are discussed. The gold standard is a combination of low molecular weight heparin and low dose aspirin as described. Another promising treatment component is hydroxychloroquine that is only to be used within SAPS. Statins showed promising results within PAPS and SAPS patients. However, the FDA did not approve the use among pregnant women. Complement inhibitors may be used when classical anticoagulants cannot be used due to contraindications. Those are considered to be the most promising for the future treatment of OAPS and APS in general. Apart from complement inhibitors, plasmapheresis and the use of intravenous immunoglobulins showed a significant lower rate of complications introducing a relatively new treatment perspective. A flow diagram of therapeutic approaches to the obstetric antiphospholipid syndrome is included in the thesis in order to facilitate a clear treatment regime. Apart from the pharmaceutical treatment it must be stressed that pre-conceptional counselling should be conducted in every case.

This thesis summarizes the recent findings focussing mainly on the mechanism in which the existence of aPl leads to pregnancy complications, diagnosis and treatment of OAPS as well as APS in general. The results of this literature research confirm the existing believe that there is a strong connection between aPl and negative pregnancy outcomes.

In my opinion, further research especially regarding mechanism how aPl lead to pregnancy complications is needed for an improved maternal and neonatal outcome. As we all know almost all medications have side effects. Especially within pregnant women extensively studied treatment must be used in order to prevent damage to an unborn child. It is important that future clinical studies include a great number of patients with standardized inclusion criteria. This will help to prevent errors and deviations between results. In my personal experience

a variety of people working in the medical field encounter APS patients. However, this disease if mentioned at all is only briefly discussed in medical schools. I hope that my thesis and other works are used in order to expand the knowledge within the people working in the medical field. This may lead to the earlier detection of APS and OAPS within women preventing the psychological damage resulting in e.g. recurrent early pregnancy losses.

9 Literature

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