Maternal recognition of the (semi) allogeneic fetus during implantation and pregnancy

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Chapter 3

Beneficial or harmful effect of anti-paternal human leukocyte antibodies on pregnancy outcome? A systematic review and meta-analysis

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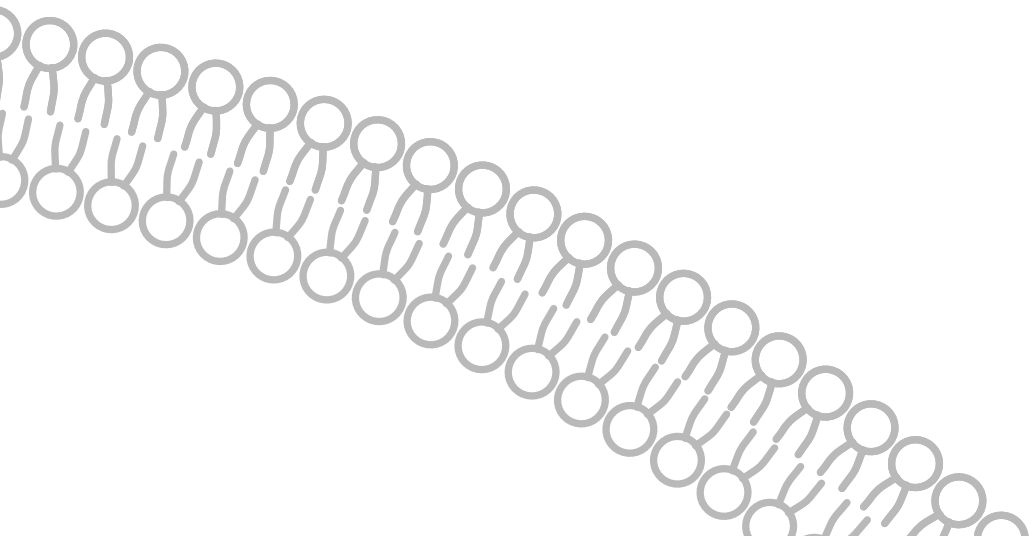


Abstract

Problem

During pregnancy antibodies are induced that target the paternal human leukocyte antigens of the semi-allogeneic fetus. The level and presence of these antibodies have been reported elevated as well as decreased for a variety of pregnancy complications; the clinical relevance and consequences of these antibodies is not very clear. Therefore, the objective of this review is to determine whether the presence of anti-paternal antibodies influences pregnancy outcome.

Method

We performed a systematic search of studies that described the effect of anti-paternal antibodies on pregnancy complications. The primary outcome was the risk ratio for HLA Class I and Class II antibodies on pregnancy complications. Furthermore, we calculated the risk for first and third trimester complications.

Results

The seventeen studies which were selected for meta-analysis showed high level of statistical and clinical heterogeneity. In the meta-analysis we found no significant effect of HLA Class I or Class II antibodies on pregnancy outcome.

Conclusions

No consistent conclusions can be drawn from the meta-analysis. Discrepancies in the meta-analysis are the result of different screening techniques, varying time points of screening and use of incorrect control groups. Furthermore, more detailed analyses of the characteristics and specificity of the antibodies involved are essential.

Introduction

During pregnancy the maternal immune system has to tolerate the persistence of semi-allogeneic fetal cells in maternal tissue, both locally at the fetal-maternal interface, as well as systematically. The entry of fetal material into the maternal circulation exists as microparticles are released from the syncytiotrophoblasts and shed into the maternal peripheral blood [197,198]. Furthermore, fetal cells (microchimerism) [2], fetal DNA, and debris from apoptotic cells flow into the circulation. The presence of these fetal antigens, as well as processing and presenting of major histo-compatibility complex (MHC) alloantigens by macrophages, enables fetus-specific antigen recognition by the maternal adaptive immune system. Indeed, primed T cells to paternal HLA antigens and fetus-specific minor histocompatibility complexes, like HY, have been demonstrated in the peripheral blood of pregnant women [169-171]. In addition, studies by our group show that the CD4+CD25dim (activated) T cell population increases in maternal peripheral blood during pregnancy [199].

B cell activation occurs after uptake of antigen by the B cell receptor, followed by interaction with the primed T cells and costimulation through CD40L-CD40, producing anti- paternal antibodies [6]. Anti-paternal antibodies have been first described by van Rood et al [200] and Payne et al [201] in 1958. These antibodies are developed in 10-30% of healthy women during pregnancy [164] and their incidence is increased after 28th week of pregnancy [202]. The number of pregnancies enhances the prevalence of HLA antibodies, although the result of the immunizing effect appears to be the strongest in the first two pregnancies [203].

The immunogenicity of the paternal HLA antigens during pregnancy is affected by the HLA antigens of the mother [204] and more recent studies showed that the induction of antibodies to paternal HLA is directly correlated with the number of foreign epitopes, triplets of amino acid sequences accessible for antibodies on the paternal HLA antigens[205,206]. There is a loss of detectable antibodies months to years after immunization [171], though primed cytotoxic T lymphocytes specific for these antigens can persist for more than 10 years, even if the antibodies have disappeared [207].

A number of important clinical effects of HLA antibodies have been clearly established in transplantation and transfusion settings, including acute and chronic graft failure [208], platelet transfusion refractoriness [209] and transfusion-related acute lung injury (TRALI) syndrome [210]. For pregnancy however the clinical relevance of these antibodies is controversial; both the presence and absence of HLA- or paternal antibodies have been described in the context of pregnancy complications. Moreover, increasing evidence suggests a role of anti-paternal antibodies in the pathogenic mechanism of pregnancy

complications such as preeclampsia [211] and recurrent miscarriage [212]. We therefore reviewed the literature to determine the effect of presence of anti-paternal antibodies on pregnancy outcome.

Method

Criteria for eligibility

A search in the National Centre for Biotechnology Information Pubmed database was performed in June 2012 using the Medical Subject Headings (MeSH) terms 'HLA antigens', 'antibodies' and 'major histo-compatibility complex' in combination with 'pregnancy complications' and 'pregnancy outcome'. As a search limit English-language publications and human research was used. Additional articles were identified from the Embase and Web of Science databases. Detailed search strategy is displayed in Appendix 1.

We selected all reports with data of HLA antibodies in respect to pregnancy complications. The pregnancy complications studied were preeclampsia, recurrent miscarriage, placental abruption, gestational diabetes, growth restriction and preterm labor. These various complications are defined in Appendix 2. The definition of presence of HLA antibodies was based on the criteria used by the various authors. Only studies with a comparative, uncomplicated pregnant control group were included for the meta-analysis. Exclusion criteria were: case reports, letters, comments and articles focusing on anti-paternal antibodies after paternal leukocyte immunization.

Quality assessment

Two authors (E.L. and T.M.) independently assessed for inclusion all potential studies of the search strategy. These authors also assessed quality and risk of bias, according to the Newcastle-Ottawa scale (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp maximum score 9 points), and carried out data extraction (Appendix 3). Disagreement was resolved by consensus. Low methodological quality was not an exclusion criterion.

Data analysis

The following data were extracted from the reports for the meta-analysis: design of the study, definition of the pregnancy complication, total numbers of pregnancies, births and miscarriage, number of patients and healthy controls in the study, number of patients and controls with HLA antibodies, the technique and timing of screening for anti-paternal antibodies, the ethnicity and the specificity of the antibodies. We contacted the authors if the variables were missing. When available, history of blood transfusion or transplantation was also noted.

The primary outcome of the meta-analysis was the pooled risk ratio for HLA Class I and Class II antibodies on pregnancy complications. In further analyses we calculated the pooled risk for first and third trimester complications. Furthermore, we expected statistical heterogeneity, so we used the random effects model for the meta-analysis; the heterogeneity was calculated with the I^2 statistics. This, with all of the other analyses was performed with Review Manager (RevMan) Version 5.1. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2011.

Results

Literature search

The main search identified 332 potentially relevant studies. Figure 1 shows the flow chart, leading to the final 17 references included in this review. A total of 316 studies were not relevant for the research question and were excluded. One study was identified in the reference list of an excluded article and was added to the final number.

In one study [213] two complications, placental abruption and preterm labor, were compared with uncomplicated pregnancies. All other studies focused on one pregnancy complication. The design of the seventeen studies included [168,213-228] was a case control study. Only three groups mentioned the ethnicity of participants which was Hispanic [214,215] and Italian respectively [221]; one study only stated that the participants and controls came from the same geographic area [219]. Different techniques for detecting HLA antibodies were used; the standard NIH complement dependent cytotoxicity (CDC) assay [229] detected antibodies in twelve studies [168,218-228], the enzyme-linked immunosorbent assay (ELISA) was the technique used in three studies [168,213,217]. In one study both the CDC assay and the ELISA was applied [168], Coulam et al [222] made use of the standard CDC and a complement dependent cytotoxicity assay with ^{51}Cr release. Finally, flowcytometry was used in three studies [214-216]; Bartel et al [216] used both flowcytometry and Luminex.

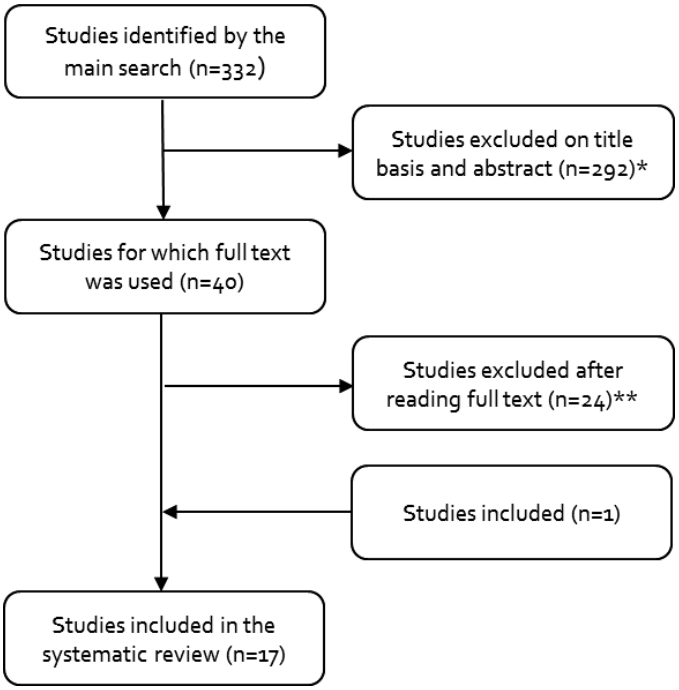


Figure 1. Flow chart depicting selection of articles for systematic review

* Major causes for exclusion were: no determination of HLA antibodies, articles focusing on paternal leukocyte immunization, articles focusing on techniques of screening, articles focusing on solid organ transplantation and case reports, letters or comments

** Major causes for exclusion were: non-comparative studies and no well defined complication group

For antibody detection and specificity determination a panel with HLA typed individuals was used for five studies though the size of these panels differed (Appendix 3). The CDC assay was tested against only paternal lymphocytes in five studies [218,222,224,226,228], both paternal lymphocytes as a panel of donors were used in the study of Jenkins et al [220] and no information was present in the study of Sargent et al [225] and Shankarkumar et al [227]. HLA positivity was differently defined (Appendix 3) within all the study groups, though the same definition (and technique) was used for both patients as controls.

Nine studies not only detected HLA antibodies, but also determined the specificity of these antibodies [168,214-217,219,221,225]. Finally the timing of measurement differed among the studies (Appendix 3); for four studies the moment of detection was not described [218,222,227,228], for two studies this was only described for the control group [221,226].

Earlier blood or tissue transplant was mentioned only in the study of Nielsen *et al* [168] and Sargent *et al* [225] as an exclusion criterion.

In Appendix 3 the assessment of methodological quality according to the Newcastle-Ottawa scale is summarized. Five studies showed high quality scoring seven or six points out of nine [168,214,215,218,222]. Mediate quality was scored by three studies [216,217,228] and nine studies [213,219-221,223-227] scored four points or less, indicating low quality.

Meta-analysis

To check for the existence of publication bias a funnel plot was drawn, which showed a symmetrical shape, indicating the absence of publication bias (data not shown). From all included studies we calculated the Odds Ratio (OR) with its accompanying standard error (Table 1). The odds ratio from the seventeen studies with class I antibodies ranged from 9.00 to 0.04; this was plotted in Figure 2. In the study of Souza *et al* [228] no HLA antibodies were detected in patients or controls; the OR could therefore not be determined.

	+ HLA antibodies		- HLA antibodies		Odds Ratio
Study or Subgroup	Events	Total	Events	Total	M-H, Random, 95% CI
Souza et al (2002)	0	0	9	18	Not estimable
Sargent et al (1988)	0	1	27	36	0.12 [0.00, 3.07]
Fujisawa et al (1985)	4	4	7	14	9.00 [0.41, 198.21]
Jenkins et al (1977)	0	7	27	41	0.04 [0.00, 0.66]
Biddle et al (1987)	8	9	55	77	3.20 [0.38, 27.11]
Shankarkumar et al (2011)	5	10	45	90	1.00 [0.27, 3.69]
Agrawal et al (2002)	9	15	96	105	0.14 [0.04, 0.49]
Steinborn et al (2004) PB	4	17	25	72	0.58 [0.17, 1.96]
Nielsen et al (2010)	30	34	39	59	3.85 [1.19, 12.45]
Umapathy et al (2011)	21	25	59	105	4.09 [1.31, 12.76]
Steinborn et al (2004) Ab	8	21	9	56	3.21 [1.03, 9.98]
Steinborn et al (2006)	12	25	35	84	1.29 [0.53, 3.17]
Bolis et al (1987)	8	67	18	204	1.40 [0.58, 3.39]
Bartel et al (2011)	15	46	97	162	0.32 [0.16, 0.65]
Coulam et al (1992) RM	160	187	449	465	0.21 [0.11, 0.40]
Kishore et al (1996)	22	71	57	108	0.40 [0.21, 0.75]
Lee et al (2011) CCA	70	106	30	144	7.39 [4.18, 13.05]
Lee et al (2011) PT	68	113	72	167	1.99 [1.23, 3.24]
Total (95% CI)		**758**		**2007**	**1.02 [0.52, 2.01]**
Total events	444		1156		

Heterogeneity: Tau² = 1.55; Chi² = 130.74, df = 16 (P < 0.00001); I² = 88%
Test for overall effect: Z = 0.07 (P = 0.94)

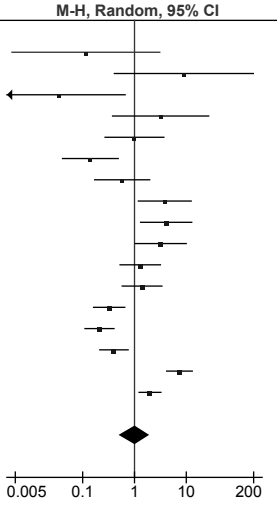


Figure 2. Meta-analysis of studies on the risk of HLA Class I antibodies with pregnancy complications
(Events = number of patients with complication, Total = number of patients and controls)

Study	*Year of publication*	*Complication*	*Participants (n)*	*OR (95% CI) HLA Class I antibodies*	*OR (95% CI) HLA Class II antibodies*
Lee et al [214]	2011	**CCA**	Patients 100, Controls 150	7,39 [4,18- 13,05]	2,81 [1,56- 5,09]
Lee et al [215]	2011	**Preterm labor**	Patients 140, Controls 140	1,99 [1,23- 3,24]	1,10 [0,61- 1,99]
Nielsen et al [168]	2010	**RM**	Patients 69, Controls 24	3,85 [1,19- 12,45]	0,8 [0,25-2,57]
Bartel et al [216]	2011	**RM**	Patients 112, Controls 96	0,32 [0,16- 0,65]	0,20 [0,08-0,46]
Steinborn et al [217]	2006	**Gestational diabetes**	Patients 47, Controls 62	1,29 [0,53- 3,17]	3,48 [1,12-10,85]
Steinborn et al [213]	2004	**Placental abruption**	Patients 17, Controls 60	3,21 [1,03- 9,98]	
		Preterm labor	Patients 29, Controls 60	0,58 [0,17- 1,96]	
Kishore et al [218]	1996	**RM**	Patients 79, Controls 100	0,40 [0,21- 0,75]	
Fujisawa et al [219]	1985	**PE**	Patients 11, Controls 7	9,00 [0,41-198,21]	0,43 [0,06- 2,97]
Jenkins et al [220]	1977	**PE**	Patients 27, Controls 21	0,04 [0,002- 0,66]	
Bolis et al [221]	1987	**PE**	Patients 26, Controls 245	1,40 [0,58- 3,39]	0,85 [0,11- 6,87]
Coulam et al [222]	1992	**RM**	Patients 609, Controls 43	0,21 [0,11- 0,40]	
Biddle et al [223]	1987	**RM**	Patients 63, Controls 23	3,20 [0,38- 27,11]	
Agrawal et al [224]	2002	**RM**	Patients 105, Controls 15	0,14 [0,04- 0,49]	
Sargent et al [225]	1988	**RM**	Patients 27, Controls 10	0,12 [0,00- 3,07]	
Umapathy et al [226]	2011	**RM**	Patients 80, Controls 50	4,09 [1,31- 12,76]	
Shankarkumar et al [227]	2011	**RM**	Patients 50, Controls 50	1,00 [0,27- 3,69]	
Souza et al [228]	2002	**RM**	Patients 9, Controls 9	Not estimable	

Table 1. Characteristics and outcome of the 17 included studies CCA (chronic chorioamnionitis) RM (recurrent miscarriage) PE (preeclampsia)

The pooled OR was 1.02 (95% confidence interval 0.52- 2.01), showing no significant effect of HLA antibodies Class I on pregnancy complications in general. The I^2 showed high heterogeneity (88% p< 0.00001) among the studies. Pooling all pregnancy complications with different gestational ages this heterogeneity was expected. Therefore we also calculated the risk of HLA Class I antibodies for first and third trimester complications (Figure 3a and b). The pooled OR for first trimester complications was 0.66 (95% confidence interval 0.29- 1.50) with high heterogeneity (I^2= 82% p<0.00001). For third

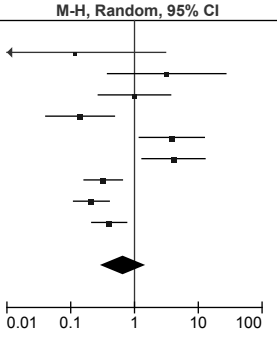
trimester complications a pooled odds ratio of 1.77 (95% confidence interval 0.83 to 3.77, I^2 = 79% p<0.0001) was calculated, showing no effect of HLA Class I antibodies on late pregnancy complications and high heterogeneity.

Moreover, we calculated the pooled risk for HLA Class II antibodies on pregnancy complications. Seven studies [168,214-217,219,221] screened and detected Class II antibodies, by flowcytometry, ELISA, Luminex or CDC (B lymphocytes). The odds ratio of Class II antibodies ranged from 3.48 to 0.2 with high heterogeneity (I^2= 80% p<0.0001); plotted in Figure 4. No significant effect was found of HLA Class II antibodies on pregnancy complications. As described above, we also calculated the OR for first trimester and third trimester complications (Figure 5a and b). The OR for first and third trimester complications was 0.37 (95% confidence interval 0.09-1.47) respectively 1.65 (95% confidence interval 0.85-3.22), showing no effect of HLA Class II antibodies on first or third trimester complications. The I^2 was 73% p=0.06 and 54% p=0.07; high to medium heterogeneity.

(a)

Study or Subgroup	+ HLA antibodies Events	Total	- HLA antibodies Events	Total	Odds Ratio M-H, Random, 95% CI
Souza et al (2002)	0	0	9	18	Not estimable
Sargent et al (1988)	0	1	27	36	0.12 [0.00, 3.07]
Biddle et al (1987)	8	9	55	77	3.20 [0.38, 27.11]
Shankarkumar et al (2011)	5	10	45	90	1.00 [0.27, 3.69]
Agrawal et al (2002)	9	15	96	105	0.14 [0.04, 0.49]
Nielsen et al (2010)	30	34	39	59	3.85 [1.19, 12.45]
Umapathy et al (2011)	21	25	59	105	4.09 [1.31, 12.76]
Bartel et al (2011)	15	46	97	162	0.32 [0.16, 0.65]
Coulam et al (1992) RM	160	187	449	465	0.21 [0.11, 0.40]
Kishore et al (1996)	22	71	57	108	0.40 [0.21, 0.75]
Total (95% CI)		**398**		**1225**	**0.66 [0.29, 1.50]**
Total events	270		933		

Heterogeneity: Tau² = 1.11; Chi² = 43.27, df = 8 (P < 0.00001); I² = 82%
Test for overall effect: Z = 0.99 (P = 0.32)



(b)

Study or Subgroup	+ HLA antibodies Events	Total	- HLA antibodies Events	Total	Odds Ratio M-H, Random, 95% CI
Fujisawa et al (1985)	4	4	7	14	9.00 [0.41, 198.21]
Jenkins et al (1977)	0	7	27	41	0.04 [0.00, 0.66]
Steinborn et al (2004) PB	4	17	25	72	0.58 [0.17, 1.96]
Steinborn et al (2004) Ab	8	21	9	56	3.21 [1.03, 9.98]
Steinborn et al (2006)	12	25	35	84	1.29 [0.53, 3.17]
Bolis et al (1987)	8	67	18	204	1.40 [0.58, 3.39]
Lee et al (2011) CCA	70	106	30	144	7.39 [4.18, 13.05]
Lee et al (2011) PT	68	113	72	167	1.99 [1.23, 3.24]
Total (95% CI)		**360**		**782**	**1.77 [0.83, 3.77]**
Total events	174		223		

Heterogeneity: Tau² = 0.78; Chi² = 33.30, df = 7 (P < 0.0001); I² = 79%
Test for overall effect: Z = 1.49 (P = 0.14)

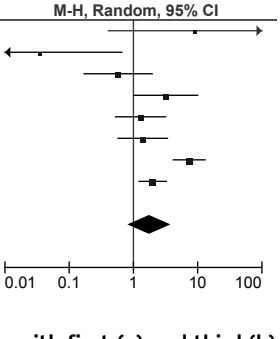


Figure 3. Meta-analysis of studies on the risk of HLA Class I antibodies with first (a) and third (b) trimester complications

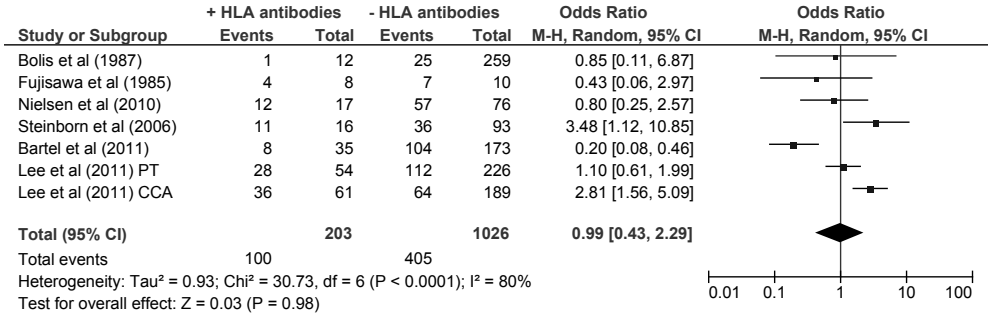
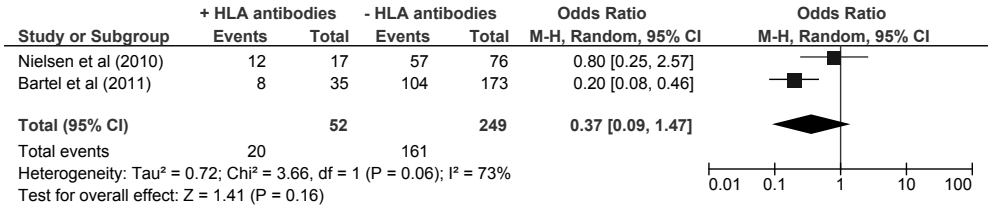


Figure 4. Meta-analysis of studies on the risk of HLA Class II antibodies with pregnancy complications

(a)



(b)

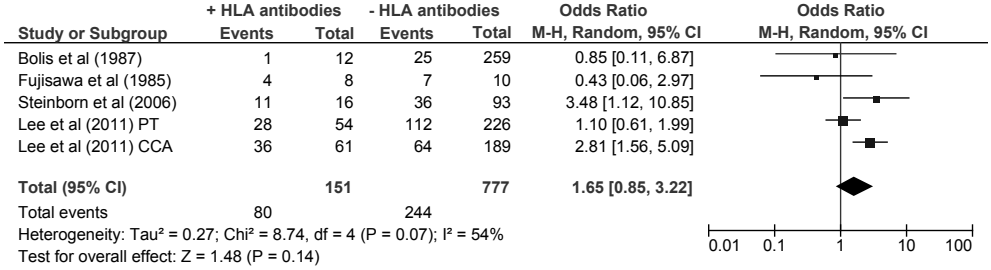


Figure 5. Meta-analysis of studies on the risk of HLA Class II antibodies with first (a) and third (b) trimester complications

Discussion

In human pregnancy the presence of antibodies against red blood cell or platelet antigen are associated with serious complications, resulting in hemolytic disease of the fetus and fetal/ neonatal allo-immune thrombocytopenia (FNAIT) respectively. In contrast with these fetal blood cell antigens, the role of HLA antibodies is not that clear as both harmful and beneficial effects have been described. In this systematic review we found no significant effect of HLA antibodies on pregnancy outcome (figure 2 and 4). The seventeen studies included however showed high statistical and clinical heterogeneity.

As we pooled all different pregnancy complications we expected a certain level of heterogeneity. Therefore, we separated the complications based on the time of onset, which resulted in a first trimester complication group and a late pregnancy complication group. The latter included chronic chorioamnionitis, preterm labor, preeclampsia, placental abruption and gestational diabetes. Though an immunological patho-mechanism is described for all of these different complications, pooling them could contribute to the reported heterogeneity. Unfortunately, too little data exist to execute a meta-analysis for each complication separately. On the other hand, the first trimester group consisted of the same pregnancy complication (recurrent miscarriage) and still showed high level of heterogeneity.

The methodological quality ranged from 3 points until 7 points maximally, with a median of 4 points which implies a low quality of the majority of studies. The largest weight in the meta-analysis was provided by a high quality study with a large patient group included.

For the meta-analysis we included data from published papers only. The funnel plot actually showed a symmetric scatter plot, which indicated no publication bias (data not shown).

The ethnicity of the participants was mentioned in only two studies. Knowledge of the race or ethnicity is of importance though as the haplotype frequencies of HLA vary amongst different populations [230] and certain combinations of fetal-maternal HLA generate significantly more antibodies [204]. Therefore it is not surprising that there is also a wide variation in incidence of anti-HLA antibodies in the sera of healthy women among different populations [226].

The timing of screening was different amongst the included studies, ranging from a gestational age of 4 weeks until 12 months postpartum. Regan et al [202] was the first to document the incidence and development of HLA antibodies measured throughout the pregnancy in a healthy population. They detected HLA antibodies only after the pregnancy has reached a gestational age of 28 weeks, probably since the influx of fetal material into the circulation peaks in the last trimester. One study included in our meta-analysis showed indeed higher IgG HLA class I and II positivity in samples obtained at time of delivery than before a gestational age of 16 weeks both for uncomplicated as complicated pregnancies [214]. Regan et al further showed that the cumulative frequency of positive HLA antibody sera increased even more after delivery, at 4 weeks postpartum [202]. This means that the highest incidence of HLA antibodies is after delivery, when fetal material entry into the maternal circulation is at maximum, in coincidence with Rhesus antibodies. Moreover, during graft rejection donor-specific antibodies may be undetectable by routine serum screening because the graft has absorbed them. After removal of the antigen source these antibodies become more readily detectable [231]. Therefore, the level of child

specific antibodies detected postpartum could be higher as a result of absorption by the placenta during pregnancy. Six studies in our meta-analysis did not mention the timing of screening for their patient and control group. In two other studies non-pregnant women were used as control group, within 2 years after their last pregnancy, while their patients were screened during pregnancy [168,224]. These differences may have impact on their results.

HLA specific antibodies formed during a previous pregnancy can be present at time of new conception [202] and studies that report a beneficial effect of HLA antibodies suggest these antibodies enhance the development of maternal-fetal immunologic tolerance. This is in line with epidemiological studies that a prior birth confers a protective effect against pre-eclampsia and IUGR [232]. Furthermore, exposure to new or different paternal antigens as a result of changed paternity is associated with an altered risk for pre-eclampsia [233]. However, it is not clear whether this beneficial effect can be attributed to the HLA antibody itself, or, for example to the period of semen exposure [234]. It is assumed that semen contains a variety of immune factors which can support the induction of maternal immunomodulation [235] and an association was found between short duration of sexual intercourse and preeclampsia [135,234]. Still, stratification according to the presence or absence of previous pregnancies is required to rule out the effect of earlier formed HLA antibodies; this was only mentioned in the study of Lee et al [214]. They observed similar results in nulliparous and multiparous women though.

For primary recurrent miscarriage, almost all the included studies used healthy multiparous women as control group. However, since the incidence of HLA antibodies in multiparous is per definition higher than in first trimester pregnancies, only nulliparous women with recurrent (≥3) elective abortions, as Biddle et al [223] stated, would reflect the appropriate control group. Interestingly, in the study of Nielsen et al [168] a subgroup of secondary recurrent miscarriages with a boy prior to the miscarriage showed a significant higher prevalence of HLA antibodies compared to healthy multiparous controls. The authors explain this higher prevalence of HLA antibodies as the result of a higher degree of microchimerism. Microchimerism occurs within 50-70% of pregnant women [236] and can persist until 27 years after delivery [237]. The traffic of fetal cells into the maternal circulation microchimerism may lead to its activation. Indeed, a reproductive history including elective termination of pregnancy [238] or early fetal loss [239] is associated with a higher incidence of microchimerism in maternal tissues. Also for preeclampsia a significant increase of fetal erythroblasts was seen compared to normotensive pregnant women [240,241], as with intra-uterine growth restriction [242,243]. This enhanced cell trafficking however may be a result of the pathological condition of the placenta rather than as a cause for these complications. A higher level of HLA antibodies may thus be just a result of higher level of microchimerism, without a causal link and without any clinical

relevance. Furthermore, regarding the study of Nielsen et al, no data exists about the kinetics of appearance of HLA antibodies around abortion or at 2 years post delivery, the time points of screening in their study.

To detect HLA antibodies five different techniques were used: the standard NIH complement dependent cytotoxicity (CDC) assay, the enzyme-linked immunosorbent assay (ELISA), complement dependent cytotoxicity assay with ^{51}Cr release, Luminex and flowcytometry. The CDC assay is based on complement activation and requires immunoglobulins that bind to complement. Immunoglobulins that are not bound will thus not be detected [244]. The indirect immunofluorescence method (flowcytometry) is independent of complement fixation and can detect all antibodies. This technique is used in the studies of Lee et al and Bartel et al [214-216]. Furthermore, the CDC assay uses lymphocytes as target for HLA antibodies, which implies that also other irrelevant cell membrane structures may be targets for antibody reactivity as well [245], possibly overestimating the actual level of HLA antibodies. To increase the sensitivity and specificity of HLA antibody detection, Luminex and flowcytometric assays are introduced that use isolated HLA molecules on beads. Indeed, Lee et al and Bartel et al [214,216,246] made use of these single-antigen beads. With the binding of HLA molecules to microspheres however a conformational change in the HLA molecule may occur, unmasking 'hidden' epitopes, which may lead to the detection of unexpected antibody reactivity [247,248]. Furthermore, whether the detected antibodies are directed against the paternal HLA of the fetus was not mentioned [215,216], or only for certain cases [214]. This child-specificity was also not mentioned in studies that used the CDC assay serum with lymphocytes from a panel of donors [219,221,223], with the exception of the study by Nielsen et al. Using paternal lymphocytes as donor to determine the extent of immunization will generate only child-specific antibodies.

The question remains nevertheless, what the clinical relevance of these (non) child-specific antibodies is. Extravillous trophoblasts, interacting with maternal cells at the decidua basalis, express only HLA-C and the non-classical HLA-E, -F and –G. Antibodies directed against these human leukocyte antigens in theory may have a detrimental effect. However, HLA-C mismatches are low in immunogenicity and the detection of HLA-C antibodies with CDC assay is not reliable; the expression of HLA-C on nucleated cells is lower than HLA-A or –B and there is linkage disequilibrium with HLA-B antigens [249]. In fact, all IgG type HLA antibodies are able to cross the placenta; HLA antibodies were demonstrated in the fetal circulation [250] and neonatal thrombocytopenia by maternal HLA antibodies was reported as side effect following leukocyte immunization in a woman with recurrent miscarriages [251].

The clinical relevance of HLA antibodies is also a matter of debate in transplantation setting [244]. Preceding organ transplantation, screening for HLA antibodies is performed as routine. The detection of donor specific HLA antibodies with CDC was considered a contraindication for transplantation, as these antibodies were associated with graft failure after transplantation [208], With the introduction of newer screening techniques the sensitivity is increased, however to determine the individual risk factor for a patient before transplantation appears impossible; the debate concerning interpretation still continues [244].

Conclusion

In this systematic review and meta-analysis we found no significant effect of HLA antibodies class I and/or II on pregnancy complications. Notably, the included studies showed high heterogeneity, both clinical as statistical. The discrepancies in the meta-analysis are the result of different screening techniques, varying time points of screening and use of incorrect control groups. Furthermore, analyses of the characteristics of the antibodies involved are essential; these include their (child) specificity, capacity to fix complement, their titer and the HLA epitopes [206] recognized. Large, observational studies, considering these characteristics are necessary to determine whether there is a beneficial or harmful effect of HLA antibodies, or that it is simply an epiphenomenon of any pregnancy.