Treatment with intravenous immunoglobulin in patients with recurrent pregnancy loss

An update

Christiansen, Ole B; Kolte, Astrid M; Krog, Maria C; Nielsen, Henriette S; Egerup, Pia

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Treatment with Intravenous immunoglobulin in patients with recurrent pregnancy loss: an update

Ole B. Christiansen[a], Astrid M. Kolte[b], Maria C. Krog[b], Henriette S. Nielsen[b], Pia Egerup[c]

[a] Recurrent Pregnancy Loss Clinic, Department of Obstetrics and Gynaecology, Aalborg University Hospital, Aalborg, Denmark

[b] Recurrent Pregnancy Loss Unit, Fertility Clinic 4071, Rigshospitalet, Copenhagen University Hospital, Denmark

[c] Department of Clinical Oncology, Zealand University Hospital, Roskilde, Denmark

Highlights are

- the results of sensitivity analysis of meta-analyses of RCTs in the treatment of recurrent pregnancy loss showing a significant therapeutic effect when treatment is started pre-conception and
- not previously published data from a follow-up study of IVIg treatment in their next pregnancy of patients who miscarried when they participated in our RCT of IVIg in their former pregnancy. These data show that IVIg treatment in a previous pregnancy seems to increase the chance of live birth 10 fold in these patients with an average of 6 previous pregnancy losses.

Abstract

Intravenous immunoglobulin (IVIg) has a documented clinical effect in many autoimmune diseases and has so far been tested in > 10 randomised controlled trials (RCTs) in women with recurrent pregnancy loss (RPL). The results of the RCTs have, however, been very

divergent. In meta-analyses of all trials, no significant impact on live birth rate has been reported. In contrast, in sensitivity analyses, IVIg significantly increased live birth rates when initiated prior to conception and it had a borderline significant therapeutic effect in women with secondary RPL. Higher dosages of IVIg and serological signs of autoimmunity in the treated patients tended to increase the success rate after treatment. A follow-up study of patients form our recent RCT also supports a significant therapeutic effect in patients who had received IVIg before conception. The lessons learned from the published trials and meta-analyses should be incorporated in the design of future RCTs of IVIg in the treatment of RPL.

Introduction

Recurrent pregnancy loss (RPL) has traditionally been defined as three or more consecutive pregnancy losses (miscarriages before gestational week 23 and biochemical pregnancies). Some societies of reproductive medicine, however, define RPL as two or more pregnancy losses (Practice Committee of ASRM, 2013; The ESHRE Guideline Group on RPL, 2018). RPL affects 1-5% of all women trying to conceive depending on the definition used. Primary RPL refers to a series of pregnancy losses without a previous birth whereas secondary RPL refers to a series of pregnancy losses subsequent to a previous live birth or stillbirth. In only a minority is the condition associated with parental chromosomal abnormalities, uterine malformations, infectious, endocrine or thrombophilic disturbances. In some cases can the condition be explained by repeated chromosomal abnormalities in the embryos of parents with normal chromosomes. In primary RPL the frequency of embryonal chromosomal abnormality has been reported to be about 55 % and in secondary RPL about 35 % (Coulam et al., 1996).

Immunological disturbances are hypothesized to play an important role in RPL since many immunological biomarkers can be found with increased prevalence in women with RPL and display a negative prognostic impact (Kruse et al., 2002; Hiby et al., 2008; King et al., 2010; Thangaratinam et al., 2011.) However, the exact pathophysiological mechanisms behind RPL still need clarification.

Various types of immune-based therapies have been tested in women experiencing RPL including intravenous immunoglobulin (IVIg) (Christiansen et al., 1992; The German RSA/IVIG group, 1994; Christiansen et al.,1995). IVIg formulations are made by extracting the IgG fractions from plasma from normal blood donors and are safe to use because of preventive virus screening and inactivation procedures and transmission of infections (e.g., HIV, hepatitis) by IVIg transfusion has not been reported for decades (Späth and Kempf, 2004).

High dose IVIg (e.g. 0.4g/kg for 5 days) is an established treatment in many autoimmune and inflammatory diseases with a large number of proven or suggested mechanisms of action. Some implicated mechanisms of action in autoimmune diseases are: interaction with Fc-receptors, inhibition of cell adhesion, inhibition of formation of/or elimination of immune complexes, interference with antigen presentation, effects on cytokines including neutralization of inflammatory cytokines, effects on apoptosis, suppression and neutralization of autoantibodies, attenuation of natural killer (NK) cells, and expansion of regulatory T lymphocytes (Negi et al., 2007; Clark et al., 2008; Baerenwaldt et al., 2010; Schwab and Nimmerjahn, 2013). Suppression of NK cell cytolytic activity by IVIg seems to be partially mediated by the CD200 tolerance signaling molecule acting on non-NK cells, which then act on NK cells by direct contact (Clark et al., 2008), In vitro, different brands of IVIg showed significant differences in the potency in suppression NK cell cytolytic activity.

So far, 9 randomised placebo-controlled trials (RCTs) investigating IVIg in women with RPL have been published with conflicting results (The German RSA/IVIG group, 1994; Christiansen et al.,1995; Coulam et al., 1995; Perino et al.,1997; Stephenson et al., 1998; Jablonowska et al., 1999; Christiansen et al., 2002; Stephenson et al., 2010; Christiansen et al., 2015). The last Cochrane systematic review with meta-analysis was updated in 2014 (Wong et al., 2014) and found overall no significant beneficial effect of IVIg over placebo in improving the live birth rate after RPL. However, this review only included one outcome (live birth after 20th weeks of gestation) and a single subgroup analysis (trials reporting intention to treat), and it did not include the most recent placebo-controlled trials of IVIg in RPL (Stephenson et al., 2010, Christiansen et al., 2015).

In this survey, we review the evidence from our own and other relevant studies for a possible treatment effect of IVIg in RPL. The focus will be to identify subsets of women

with RPL who will benefit most by IVIg treatment and identify treatment protocols, which will be most efficient.

Primary versus secondary RPL.

A priori, there is some evidence that the immunological disturbances characterizing primary and secondary RPL are different. Increased numbers and activity of peripheral blood NK cells have been reported in primary RPL compared with secondary RPL (Shakhar et al., 2003; Kuon et al., 2017) whereas increased plasma levels of inflammatory cytokines and immunity against male-specific minor histocompatilbility (HY) antigens seem to characterize secondary RPL (Piosek et al., 2013; Nielsen et al., 2009). Different effects of IVIg in the two RPL subsets are therefore expected. The systematic review and meta-analysis by Hutton et al. (2007) found that the treatment effect of IVIg was significant in secondary RPL but not in primary RPL. We therefore found it relevant to perform an updated meta-analysis with separate analyses of primary and secondary RPL, respectively.

In the systematic review and meta-analysis (Egerup et al., 2015), 11 relevant RCTs were identified involving unexplained RPL patients; nine of them compared IVIg to placebo and two RCTs compared IVIg to other treatments. We aimed to retrieve Individual Patient Data (IPD) from the original authors to be able to do relevant subgroup analyses with high statistical power but were only successful to obtain IPD from 5 of the studies. In all included patients, a relative risk (RR) of 0.92 (95% CI 0.75-1.12) for *no live birth* (and a RR of 1.02 [95% CI 0.70-1.48] for *live birth* in patients that could be classified as either primary or secondary RPL in the publications) (Fig. 1) was found after IVIg. In patients with secondary RPL, the RR for *live birth* after IVIg was 1.24 (0.96-1.59 ; p = 0.06), which can be translated into a borderline significant benefit of IVIg in secondary RPL (Fig. 1). This p-value is two-tailed but since the null hypothesis was that IVIg does not improve pregnancy success rate in secondary RPL in can be argued whether a one-sided test should be applied instead and the p-value would then be 0.03.

Wang et al. (2016) published a similar systematic review and meta-analysis on the effect of IVIg in RPL excluding two RCTs with no placebo group, which were included in the

meta-analysis by Egerup et al., but including two Chinese RCTs. The outcome measure was live birth. The meta-analysis found that IVIg treatment was associated with a RR for birth of 1.26 (95% CI 0.99-1.60; p = 0.06, one-sided test p = 0.03) in secondary RPL versus RR = 0.88 (95% CI 0.71-1.08; p = 0.20) in primary RPL (Fig 1). The difference between the effect of IVIg in the two subgroups was significantly different (p = 0.02). Both meta-analyses thus agree that IVIg has a borderline significant therapeutic effect in secondary RPL and this subset of patients must be considered the main target group for further RCTs.

As pointed out, two RCTs (Triolo et al., 2003; Mahmoud et al., 2004) were included in the Egerup et al. (2015) meta-analysis but not in the meta-analysis by Wang et al. (2016) whereas two RCTs (Liu and Chen, 2010; Lin and Li, 2015) were included in the latter but not the former meta-analysis. Since neither of these RCTs provided information separately for patients with primary or secondary RPL, combining all primary or secondary RPL patients, respectively, from the two meta-analyses is not feasible; such an analysis will comprise the same RCTs shown in Fig. 1. However, in Fig. 2 and Fig. 3 we have performed an analysis of all patients in the 13 RCTs included in the two meta-analyses. Data are shown as extracted from the original publications by Wang et al. (2016). Our own data extraction from the same original RCTs provides slightly different results. Two Forest plots are shown: in Fig. 2 the RCTs are combined by the *random effects method*, which is the more conservative method in which the group means are a random sample from a population. In this analysis, the combined RR for live birth in IVIg-treated versus placebo-treated patients is 1.17 (95% CI 0.95-1.44; p = 0.14). Performing the analysis using the *fixed models method* (Fig. 3) in which the group means are fixed, it is found that the RR for live birth in the IVIg-treated patients is significantly higher than in the placebo-treated patients (RR = 1.20; 95% CI 1.06-1.37; p = 0.004). However, since significant heterogeneity is found when comparing RRs of the 13 RCTs, the analysis by the *random effects method* has highest validity.

Low versus high dosages of IVIg

In the treatment of several autoimmune diseases, IVIg protocols providing 0.4 g/kg body weight for 5 consecutive days are standard doses with documented clinical effect (Imbach, 2004). None of the RCTs of IVIg in RPL has administered dosages approaching these doses. In one of the RCTs by Christiansen et al. (2002), 0.8 g/kg body weight each week was given; which is the highest dose given in any RCT. In almost all published trials, the protocol prescribed 0.4g/kg body weight with 2-3 weeks intervals. In our meta-analysis (Egerup et al., 2015) we looked at the efficiency of IVIg in RCTs providing dosages above versus below the median dosage of 84 g IVIg until gestational week 10 in all RCTs. The RR for *no live birth* (= miscarriage) in the high dosage group was 0.85 (95% CI 0.64-1.12) compared with RR 1.19 (95% CI 0.81-1.75) in the low dosage group, the difference between RRs was not significant (p = 0.17). The trend for higher treatment efficiency with the higher doses should be explored in future studies. However, in a pilot study from the RPL clinic at Copenhagen University Hospital, a substantial increased risk of side effects especially severe headache was experienced using a protocol with four weekly infusions of IVIg.

Patients with autoantibodies versus no autoantibodies

IVIg has a documented effect in a series of diseases with an autoimmune background such as idiopathic thrombocytopenic purpura, Guillain-Barré syndrome and myasthenia gravis often characterized by the presence of autoantibodies. It is therefore natural to suggest that it will exhibit higher efficiency in women with RPL positive for autoantibodies compared with those without. In a cohort of 52 women with RPL after IVF/ICSI treatment who were treated with a combination of IVIg and prednisone (10 mg daily) from the start of the next IVF/ICSI cycle (Nyborg et al., 2014), the cumulative live birth rate after up to 5 IVF/ICSI attempts with immunomodulation was 68.4% in patients positive for at least one of the autoantibodies routinely investigated in the RPL Unit at Copenhagen University Hospital (IgG anticardiolipin, antithyroid-peroxidase, antinuclear, anti-ds-DNA antibodies and lupus anticoagulant) compared with 57.6% in those completely negative for the autoantibodies (p = 0.44). Although the difference was not statistically significant, the 11% better outcome in the autoantibody-positive patients calls for further studies on immunological biomarkers that can identify a patient group, which benefits from IVIg. In

addition to autoantibodies, it would be of particular interest to study whether patients with high natural killer (NK) cell count or cytotoxicity in the blood or endometrium constitute a target group for IVIg therapy since numerous studies have shown that IVIg treatment of women with RPL reduces these NK-cell related parameters (Roussev et al., 2007; Moraru et al., 2012) and the reduction of NKT or NK cell numbers after IVIg may increase the live birth rate (van den Heuvel et al,, 2007; Heilmann et al., 2010).

Pre-conception versus post-conception IVIg treatment

Implementation of some of the effects of IVIg on immunological interactions takes time and therefore starting infusions only when the pregnancy test is positive may, in principle, be too late to prevent a new pregnancy loss in women with RPL in the early first trimester. Hutton et al. (2007) in their meta-analysis did several sensitivity analyses and found that in RCTs of IVIg in RPL where infusions were started before conception, the outcome in the treated patients was significantly better than in those who received placebo whereas no treatment effect could be found in RCTs starting infusions after conception (Fig. 4). In the meta-analysis by Wang et al. (2016) including a higher number of RCTs similar findings were reported (Fig. 4). The RR for live birth in patients who received IVIg before conception versus those who received placebo was 1.69 (95% CI 1.33-2.14; $p < 0.0001$). However, it should be noticed that there was substantial overlap between the RCTs analyzed by Hutton et al. (2007) and Wang et al. (2016).

These results are in line with follow-up data from our latest double-blind RCT on IVIg in patients with secondary RPL (Christiansen et al., 2015). In this RCT, an only 5% higher live birth rate was found in IVIg-treated women with secondary RPL compared with those who received placebo. However, all patients who miscarried in the trial were offered active treatment with IVIg in their next pregnancy. Since the allocation code was not broken before 2014, the treatment in the next pregnancy was in almost all cases given without knowledge of previous treatment. Figure 5 shows outcome in the first IVIg-treated pregnancy after participation in the RCT according to the treatment received in the RCT. The patients received the first IVIg infusion in the subsequent pregnancy median 25 weeks (range 10-51 weeks) after the first IVIg infusion or median 28 weeks (range 17-121 weeks)

after the first placebo infusion in the RCT. The mean number of previous pregnancy losses and age were comparable between the two groups (Fig. 5). The live birth rate was significantly higher (RR = 9.60, 95% CI 1.44-63.77, p < 0.02) in those who had previously miscarried in spite of IVIg treatment compared with those who miscarried with placebo. The results concur with the data in Figure 4: treatment with IVIg prior to conception increases the chance of live birth. The difference between the RCTs in Figure 4 that provided IVIg before conception and our follow-up study is, that in the former trials IVIg was provided to non-pregnant patients typically few weeks or months prior to conception whereas in our follow-up study all patients had secondary RPL with a very high number of previous losses and they were pregnant at the time of the first IVIg infusion(s). In normal pregnancies fetal tissue is often recognized by the maternal T- and B-cells resulting in formation of IgG antibodies against fetal HLA or long-lasting cellular immunity against male-specific HY minor antigens (Verdijk et al., 2004). In normal pregnancies trophoblast is resistant to damage by activated T-effector cells or NK-cells but bystander damage by excessive immune inflammation in the decidua may render the trophoblast susceptible to damage by T- and NK cells, which may result in pregnancy loss and RPL (Mellor and Munn, 2006) The role of IVIg in prevention of RPL may be to reduce this decidual inflammation.

Decidual inflammation may be partially regulated by T-regulatory cells (T-regs) in the decidual tissue and uterine lymph nodes. This recruitment of T-regs may be promoted by estrogen and seminal antigens in the uterus already at the time of conception (Robertson et al., 2018) but further selection and commitment of T-regs probably take place after implantation. The T-reg response is enhanced by the presence of dendritic cell, which promote a T-reg response. The CD200 check point inhibitor (present in IVIg) seems in murine studies to activate dendritic cells, which may enhance the T-reg response when given before pregnancy and reduce pregnancy loss rate (Gorczynski et al., 2002). Other mechanisms of induction of T-regs by IVIg have been reported (Trinath et al., 2013)

The seemingly higher therapeutic effect of IVIg in our follow-up study compared with the RCTs where IVIg was initiated prior to conception (RR for live birth 9.60 vs 1.67-2.39; Fig 5, Hutton et al., 2007; Wang et al., 2016) may from an immunological point of view be explained by hypothesizing that T-regs reducing inflammation in the decidua are being

positively selected in uterine lymph nodes in the presence of high IVIg concentrations (Trinath et al., 2013). Relevant immunological investigations in women with RPL before and after IVIg treatment are needed to clarify this issue, however, the most important investigations of immune competent cells in the uterine lymph nodes and endometrium pose severe methodological challenges.

Conclusion

In meta-analyses of all RCTs performing no sensitivity analyses, the therapeutic benefit of IVIg in the prevention of RPL is non-significant. Our subgroup analyses suggested that women with secondary RPL may potentially be more responsive to IVIg when compared to women with primary RPL. Furthermore, initiating IVIg before conception seems to increase the live birth rate whereas no or only weak effects can be found in RCTs starting infusions during pregnancy. IVIg dosages closer to those used in autoimmune/inflammatory diseases might be more efficient than lower dosages and the treatment might be more efficient in patients positive for biomarkers associated with dysregulated immunity than in those without. In the planning of future RCTs investigating the efficiency of IVIg in RPL treatment, the lessons learned in the trials undertaken so far should be incorporated. In short, in future trials on the topic, both more methodological and biological rigor (selection according to relevant biomarkers) must be present.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Legends to figures

Figure 1: Forest plots depicting results from two meta-analyses of the efficiency of intravenous immunoglobulin (IVIg) in patients with primary and secondary recurrent pregnancy loss (RPL), respectively. In both studies , the outcome measure is *live birth.* The difference between the outcome measure in the two subgroups was statistically significant both in the Wang et al. study (p = 0.02) and in the Egerup et al. study (p = 0.03).

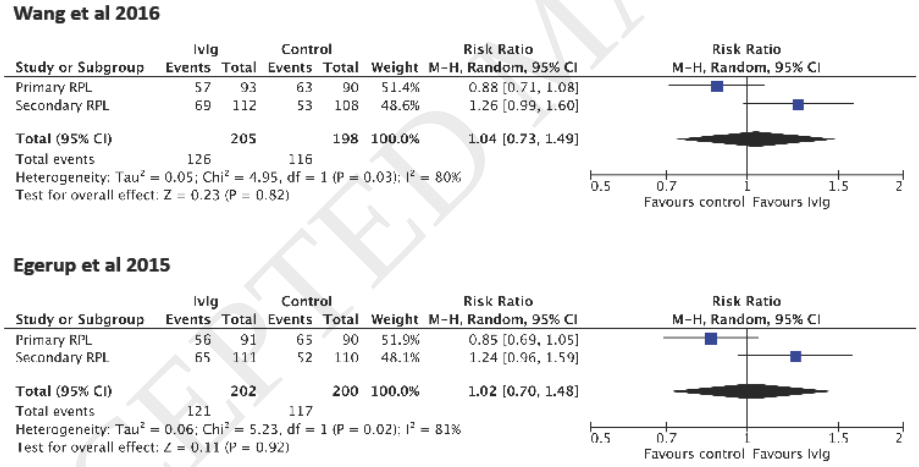

Figure 2*:* Forest plot showing the combined relative risk (RR) for *live birth* in all patients with recurrent pregnancy loss (RPL) who participated in randomized

controlled trials of intravenous immunoglobulin (IVIg) versus placebo or other treatment. Data were combined by the *random effects method.*

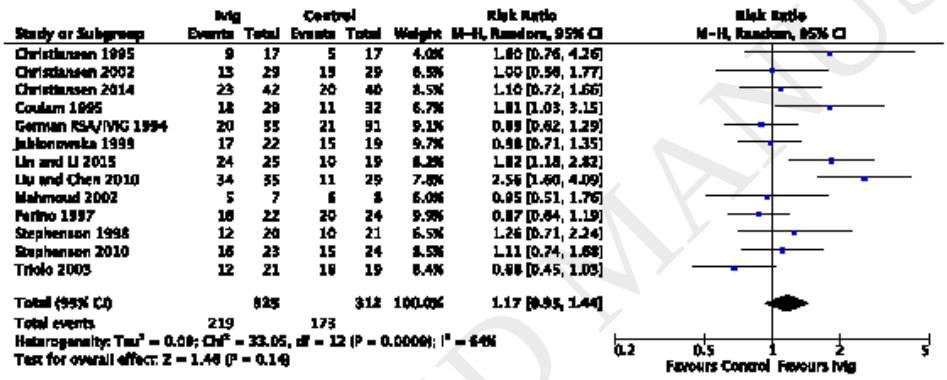

Figure 3*:* Forest plot showing the combined relative risk (RR) for *live birth* in all patients with recurrent pregnancy loss (RPL) who participated in randomized

controlled trials of intravenous immunoglobulin (IVIg) versus placebo or other treatment. Data were combined by the *fixed effects method.*

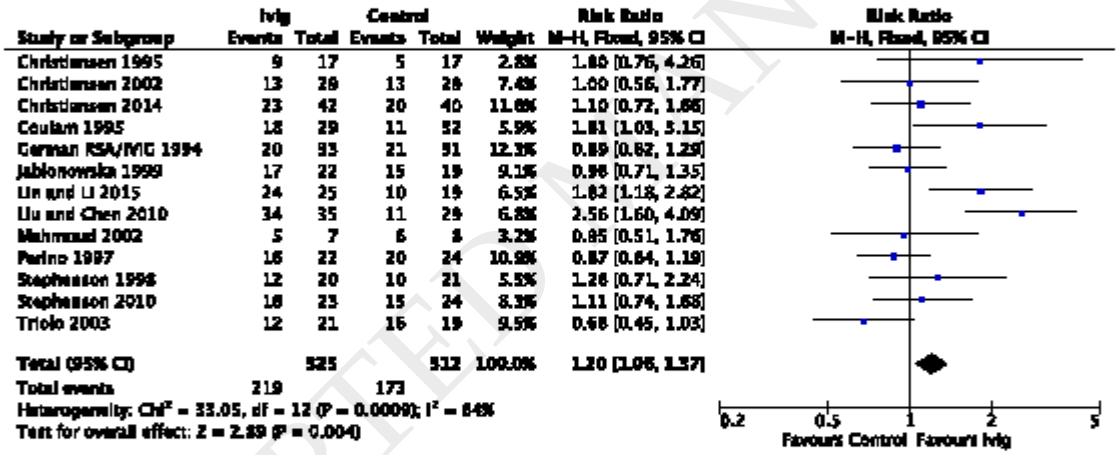

Figure 4: Forest plots from two meta-analyses of the efficiency of intravenous immunoglobulin (IVIg) in patients with recurrent pregnancy loss (RPL) who participated in randomized controlled trials where infusions were initiated before or after conception, respectively. In both meta-analyses the outcome measure was *live birth.* The differences between the outcome measure in the two subsets were statistically significant in the Wang et al. (2016) study (p = 0.005).

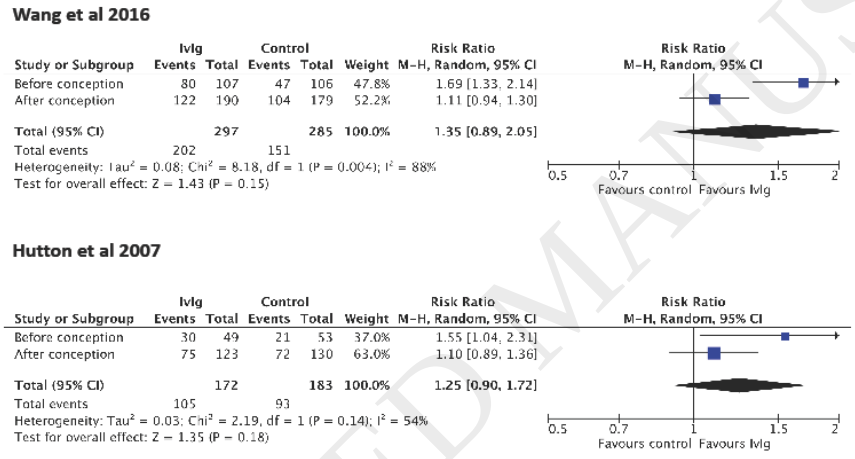

Figure 5: Outcome of the first pregnancies with infusions of intravenous immunoglobulin (IVIg) subsequent to a pregnancy loss happening when the patients participated in a placebo-controlled trial of IVIg in secondary recurrent pregnancy loss (RPL).