Heparin for women with recurrent miscarriage and inherited thrombophilia: an international multicentre randomised controlled trial (ALIFE2)

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ABSTRACT

Background

It has been hypothesized that anticoagulant therapy reduces both number of miscarriages and adverse pregnancy outcomes in women with recurrent pregnancy loss (RPL) and inherited thrombophilia.

Methods

The ALIFE2 trial was an international open-label randomised controlled trial undertaken in hospitals in the United Kingdom (n=26), The Netherlands (n=10), USA (n=2), Belgium (n=1) and Slovenia (n=1). Women (18-42 years) who had two or more pregnancy losses and confirmed inherited thrombophilia who were trying to conceive or pregnant ≤ 7 weeks gestation were eligible for inclusion. Women were randomly assigned (1:1) to use low dose, low-molecular-weight heparin (LMWH) or standard care once they had a positive urine pregnancy test. LMWH was started at ≤ 7 weeks gestation and continued until the end of pregnancy.
The primary outcome measure was live birth rate. Safety outcomes included bleeding episodes, thrombocytopenia and skin reactions. The trial was registered within the Dutch Trial Register (NTR3361) and EudraCT (UK: 2015-002357-35).

Findings: Between August 2012 and January 2021, 10,626 women were assessed for eligibility, 428 registered, 326 conceived and were randomised. 164 were assigned to LMWH and 162 to standard care. 116 (71.6%) women in the LMWH and 112 (70.9%) in the standard care group had live births (adjusted OR 1.08, 95% CI 0.65 to 1.78; absolute risk difference, 0.7%, 95% CI -9.2% to 10.6%). 39 women (23.8%) in the LMWH group and 37 (22.8%) women in the standard care group reported adverse events.

Interpretation: LMWH did not result in higher live birth rates in women who had two or more pregnancy losses and confirmed inherited thrombophilia. We do not advise use of LMWH in women with RPL and inherited thrombophilia and we advise against screening for inherited thrombophilia in women with RPL.

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Introduction

Recurrent miscarriage, defined as the loss of two or more pregnancies, affects approximately 3% of couples trying to conceive. Experiencing recurrent miscarriage can have profound impact on physical and psychological wellbeing.[1,2] Thrombophilia has been implicated in the aetiology of recurrent miscarriage, partially by the concept of thrombosis of the microvasculature of the placenta and through inhibition of extravillous trophoblast differentiation.[3] International professional guidelines recommend heparin treatment for antiphospholipid syndrome, an acquired thrombophilia which is present in approximately 15% of women with recurrent miscarriage.[4-11]. However, although inherited thrombophilia such as factor V Leiden, prothrombin 20210A mutation, and deficiencies of antithrombin, protein C or protein S have been associated with pregnancy loss,[2,12-15] guidelines do not recommend heparin treatment.[4-7] This is largely due to absence of trial evidence for this population, rather than evidence of absence of an effect.[4-7,15] Despite the absence of evidence and guidance, many clinicians prescribe heparin to women with recurrent miscarriage and inherited thrombophilia.[16] The European Recurrent Pregnancy Loss (RPL) guidelines recommended "research into the effect of anticoagulant treatment for RPL women with hereditary thrombophilia",[4,5] something that was echoed in UK guidelines and a multi-disciplinary research priority setting partnership.[6,17]

We performed an international, randomised controlled trial in women with recurrent miscarriage and inherited thrombophilia to investigate the effect of low-molecular-weight heparin (LMWH) on live birth rates, as compared to standard care.

Methods

Study design and participants

The ALIFE2 study was an international, multi-centre, open-label, randomised controlled trial to compare LMWH with standard pregnancy surveillance in women with inherited thrombophilia and a history of recurrent miscarriage. The rationale for and the design of the ALIFE2 study have been reported previously.[18] The ALIFE2 study recruited participants in the Netherlands, the UK, the USA, Belgium and Slovenia. The trial was led by two main centres, Amsterdam University Medical Centers, University of Amsterdam in The Netherlands and University of Warwick Clinical Trials Unit in the UK. The Netherlands coordinated recruitment in 14 hospitals in the Netherlands, USA, Belgium, and Slovenia. The UK coordinated 26 sites in England, Scotland, Wales and Northern Ireland. The study protocol was approved by the institutional review boards of all participating centres, and in the UK, by NRES, MHRA and HRA. Written informed consent was obtained from all participants prior to randomisation.

Women aged between 18 and 42 years at time of randomisation were eligible if they had recurrent miscarriage (≥ 2 consecutive or non-consecutive miscarriages or intrauterine fetal deaths, irrespective of gestational age), were attempting to conceive or were less than 7 weeks pregnant and had an inherited thrombophilia. Included inherited thrombophilia types were factor V Leiden mutation, prothrombin gene mutation (G20210A), antithrombin deficiency, protein C deficiency, or protein S deficiency. Antithrombin, protein C, and protein S deficiencies needed to be diagnosed by two tests, performed on two separate occasions outside pregnancy or the 6-week post-partum period. Exclusion criteria were body weight lower than 50kg, an indication for anticoagulant treatment

during pregnancy as assessed by the treating physician, contraindications to LMWH, known allergy to at least 3 different LMWH preparations and previous inclusion in the ALIFE2 study.

Recruitment, randomisation and masking

Women who were eligible for the study were recruited in recurrent miscarriage or vascular medicine/haematology clinics prior to pregnancy or before 7+0 weeks gestation. Women were informed about the study prior to pregnancy. Patients were instructed to undergo a urine pregnancy test as soon as their menstrual periods were delayed, or a pregnancy was suspected. In the UK the majority of eligible women consented and registered into the study prior to pregnancy. Participants then contacted research teams as soon as they were pregnant. If the hospital pregnancy test was positive they were randomised. In centres coordinated by the Netherlands, eligible women were recruited and informed about the trial and contacted the hospital once pregnant. They had their pregnancy confirmed, consented to the study and were randomised at the same time.

Women were randomly assigned to LMWH or no LMWH in a 1:1 ratio using two secure internet facilities for the two separate lead centres. For the Netherlands coordinated centres, randomisation was balanced in permuted blocks with maximum block size of 6 stratified for maternal age (<36 or ≥36 years), number of miscarriages (2 or ≥3) and centre type (tertiary or non-tertiary). In the UK randomisation was performed by minimisation, stratified for maternal age (<36 or ≥36) and number of prior miscarriages (2 or ≥3). There was concealment of allocation for physicians and participants. There was no masking to assigned study group for physicians or participants, as the trial design was open-label. Outcome assessors were not masked with respect to the study treatment.

Sample size calculation

The study hypothesis was that LMWH would increase the rate of live birth as compared to no LMWH. In the first ALIFE study that included women with unexplained recurrent miscarriage, the occurrence of live birth in the subgroup of women with inherited thrombophilia and who became pregnant was 60% in those who were randomised to placebo.[19] Assuming a live birth rate of at least 55% for women receiving standard care, the randomisation of 324 participants would allow the detection of an absolute difference in excess of 15% with a power of 80% and a two-sided significance level of 5%. The absolute risk difference of 15% was defined following consultations amongst health care providers and participants.[18] The UK team aimed to recruit women pre-conceptually, and the sites managed by the Netherlands aimed to recruit women once pregnant. A target of recruitment of 400 patients was set to allow for women who did not become pregnant, and also for an expected nominal degree of drop out due to non-compliance, loss to follow-up and exclusion from the study (e.g. ectopic pregnancy). This recruitment target was estimated to deliver the 324-randomisation requirement.

Procedures

LMWH consisted of prefilled syringes containing enoxaparin 40 mg, (Clexane (Sanofi-Aventis)) or (Inhixa (Techdow Pharma Ltd)), dalteparin 5000 IU, (Fragmin (Pfizer bv)), tinzaparin 4500 IU (Innohep (Leo Pharma bv)) or nadroparin 3800IU (Fraxiparin, (GlaxoSmithKline bv)); doses were not adjusted to body weight. The type of LMWH used was left to the discretion of the care-providing clinician, in accordance with regular clinical care in each of the countries. Women self-administered LMWH once a day subcutaneously. LMWH was started as

soon as possible after a positive pregnancy test and before 7+0 weeks gestation and continued throughout pregnancy. Women were instructed to discontinue LMWH when labour started. Women allocated to LMWH were discouraged from using antithrombotic or other medications that affect haemostasis, including NSAIDs. Low-dose aspirin (≤ 150 mg daily) to decrease the risk for preeclampsia was given after 10 weeks' gestation to women at increased risk of pre-eclampsia, at the treating physician's discretion and its use was recorded. All women were encouraged to take folic acid 400µg daily, starting before conception and continuing until 8 weeks after conception.

All women received standard care provided by their own obstetrician throughout pregnancy including structural fetal ultrasound evaluation at 18-22 weeks gestational age. Women were contacted by telephone at 10-14 weeks, 22-28 weeks and 34-36 weeks until completion of pregnancy by a dedicated research nurse, who assessed compliance and side effects. Side effects of bruises, nose or gum bleedings, haematuria, skin reactions at the injection sites and gastro-intestinal complaints were recorded during every contact.

Outcomes

The primary outcome measure was live birth after 24+0 weeks gestation. Secondary outcomes included incidence of and type of miscarriage (biochemical, first trimester, second trimester), ectopic pregnancy, termination of pregnancy and obstetric complications including pre-eclampsia, HELLP-syndrome, small for gestational age (defined as birth weight below 10th percentile for gestational age and sex), placental abruption and premature delivery (defined as delivery before 37+0 weeks gestation). Maternal thrombocytopenia, bleeding episodes, skin reactions and neonatal abnormalities were monitored for safety.

Statistical analysis

Analysis included all available data from all women who were randomised and did not withdraw consent to be followed up, as per the intention-to-treat principle. The primary outcome of live birth after 24+0 weeks gestation was compared across randomised treatment arms using a chi-squared test with continuity correction, and then sensitivity analysis was undertaken using logistic regression to adjust for stratification factors. Absolute risk differences with 95% confidence intervals (CI) were also calculated.[20] Prespecified exploratory subgroup analyses were performed to investigate the treatment effect within the levels of age group (<36, ≥36), number of previous miscarriages (2, ≥3), previous live birth (yes, no) and type of inherited thrombophilia. To assess whether treatment effects vary among the levels of these factors, tests for interaction were performed. Formal statistical testing of the secondary outcomes was not undertaken due to low frequencies. The planned sensitivity analyses to explore the effects of missing data were also not undertaken due to low incidences of missing data.

Role of the funding source

The study was endorsed by INVENT-VTE and funded from the Netherlands Organization for Health Research and Development (NWO, VIDI innovative research grant 016.126.364 awarded to S. Middeldorp) and UK National Institute for Health under its Research for Patient Benefit (RfPB) Programme (Grant Reference Number PB-PG-1013-32011). None of the funders had a role in the design, data collection, data analysis, data interpretation or writing of the report.

Results

Enrolment took place between 1st August 2012 and 30th January 2021 with a pause to recruitment due to the COVID19 pandemic between 24th of March and 18th of May 2020.

A total of 428 women were registered and 326 women were randomised. The trial was stopped when the planned recruitment target was reached. The consort diagram (Fig. 1) shows that in the UK 10,626 women with recurrent miscarriage were assessed for eligibility, with the most common reason for ineligibility being not having an inherited thrombophilia (90%). Figures for screening were not collected by the Netherlands managed sites and participants were randomised once pregnant. A total of 164 women were allocated to LMWH and standard care and 162 women to standard care alone. One participant was lost to follow up. In the standard care arm 30 participants received LMWH, of whom 18 started heparin treatment before 12 weeks of gestation. Twelve received LMWH after 12 weeks, 6 of whom after 28 weeks of gestation as they were assessed as needing LMWH for thromboprophylaxis, as per RCOG guidelines.[21]

There were no differences between trial arms in terms of baseline characteristics (Table 1). The mean age of the participants was 33 years, approximately one third being 36 years or older, and the majority was of Caucasian ethnicity (83%). The median number of miscarriages prior to randomisation was 3 (interquartile range 2 to 4), and 70% had a history of 3 or more miscarriages. The most common thrombophilia types were heterozygosity for factor V Leiden (56%), prothrombin 20210A mutation (25%), and protein S deficiency (13%). Aspirin was used as co medication in 11%.

Details about type of LMWH were available on 157 (95.7%) of the 164 randomised to receive it. The most commonly LMWH administered was enoxaparin (73%), followed by dalteparin (18%), tinzaparin (8%) and nadroparin (1%). Four participants had the type of LMWH changed during the trial, 2 enoxaparin to dalteparin, and 2 dalteparin to nadroparin.

Of the 326 randomised participants, 320 (98.2%) had primary outcome data available (Table 2). Live birth rates were 116/162 (71.6%) in the LMWH arm and 112/158 (70.9%) in the standard care arm. No significant difference was detected between arms with either the unadjusted (chi-squared p=0.99, odds ratio [OR] 1.04 (95% CI 0.64, 1.68)) or adjusted (OR 1.08 (95% CI 0.65, 1.78), p=0.77) analyses. The absolute risk difference between groups was 0.7% (95% CI: -9.2% to 10.6%).

There were minimal differences between randomised arms in the secondary outcome measures (Table 3). Importantly, there were very similar numbers and types of pregnancy loss and pregnancy complications in each arm. Additionally, there were no differences in reported bleeding complications in each arm and no cases of heparin induced thrombocytopenia. As expected, easy bruising was reported by 73 (45%) women in the LMWH and 16 (10%) in the standard care arm. There were no serious adverse events deemed related to the trial medication.

The results of the planned subgroup analyses of live birth rates revealed no evidence of efficacy of LMWH in any of the pre-specified subgroups, without significant interaction effect between these subgroups, with all 95% CIs overlapping (Fig 2).

A post-hoc, exploratory 'per-protocol' analysis was undertaken comparing patients randomised to and receiving LMWH with patients who were randomised to standard care and who didn't receive LMWH within the first 12 weeks, and similar results were found. No significant differences between the groups were detected in live birth rates; 116/162 (71.6%) in the LMWH group, 99/143 (69.2%) in the standard care only group, (unadjusted p=0.74, adjusted p=0.56).

Discussion

Our international open-label randomised controlled trial showed no significant difference in live birth rates in women with recurrent pregnancy loss and confirmed inherited thrombophilia after treatment with LMWH treatment when compared with standard care alone. There was also no evidence of differences in any of the secondary outcomes, including miscarriages and adverse pregnancy outcomes comprising premature delivery and small for gestational age. As expected, low-dose LMWH in pregnancy appeared to be safe; there was no increase in minor or major bleeding in those randomised to LMWH as compared to no LMWH and there were no cases of heparin-induced thrombocytopenia. However, 45% of women who received LMWH treatment reported easy bruising mainly around injection sites.

In the absence of published randomised controlled trials that assess the efficacy of LMWH therapy in women with recurrent miscarriage and inherited thrombophilia, a clinical trial that addresses this topic was highly needed. Worldwide, the clinical use of LMWH in these women in clinical practice, outside of a trial, was increasing. Recruitment to this type of trial is very difficult due to the relative rarity of women with recurrent pregnancy loss with inherited thrombophilia, problems with clinicians not being in equipoise and therefore not screening for thrombophilia or alternatively giving all screen-positive women LMWH and women with many pregnancy losses wanting a medication. We succeeded in recruiting the intended number of women, with only 6 women declining follow up, through an international collaboration and persistence. Another strength of the trial is that we used a pragmatic trial design that reflects daily clinical practice and analysed the data according to the intention-to-treat principle. The generalisability of the findings is reasonable because we included women from multiple centres and multiple countries. Most participants were from the United Kingdom, the Netherlands and some women were recruited from Belgium, Slovenia and USA. Compliance with allocated treatment was good. Whilst 30 women in the standard care arm took LMWH at some point in pregnancy, only 18 cases were in the first trimester when they could have influenced miscarriage rates.

Our trial also has potential limitations. First, the definition of recurrent pregnancy loss was broad, making it possible that women with sporadic miscarriages were included. We chose the inclusion criteria for the ALIFE2 trial to keep our study population as similar as possible to clinical practice. Furthermore, the 2017 and recently updated European ESHRE guideline "Recurrent Pregnancy Loss" states that - based on the best available evidence-, a diagnosis of recurrent pregnancy loss is to be considered after the loss of two or more pregnancies.[4,5] Having said so, it is important to note that 70% of our study population had 3 or more miscarriages and that there was no significant interaction between treatment assignment and number of miscarriages (2 or ≥3) with respect to live birth rate. Second, LMWH was initiated after the implantation phase and without ultrasound confirmation

of a viable pregnancy. We believe this is reasonable as there is no clinical trial evidence that LMWH improves implantation,[22] and we wanted to start LMWH as early as possible and avoid delays due to waiting for ultrasound scanning. The start of LMWH treatment was analogous to how LMWH is used in antiphospholipid syndrome, where there is evidence of a beneficial effect.[23] Third, different types of LMWH were used in our trial. However, it can be regarded as a reflection of daily practice and it is unlikely that the different types had any differing efficacies. We cannot exclude a 10% or less effect on live birth rate but the small, 0.7% absolute difference between the two groups, and lack of any signal in the planned subgroups suggest that any effect on live birth rate is unlikely. Finally, the lack of blinding of patients, treating physicians and outcome assessors may be a limitation. However, the primary outcome event of live birth is unlikely to be subject to diagnostic suspicion bias.

Our results answer a question posed in the literature scattered with small underpowered trials and non-randomised studies. A prospective cohort study including 126 women with a thrombophilia and pregnancy loss, found that LMWH is associated with an increased chance of live birth (OR 10.6, 95% CI, 5.0 to 22.3), concluding that LMWH was beneficial in preventing pregnancy loss.[24] Other authors suggest that LMWH would not improve live birth rates in women with pregnancy loss and thrombophilia. A meta-analysis of mainly subgroups of eight small trials comparing LMWH to no LMWH during pregnancy in women with inherited thrombophilia and heterogeneous pregnancy morbidity did not demonstrate a clinical benefit of LMWH but agreed the ALIFE2 results were needed (relative risk 0.81; 95% CI 0.55 to 1.19).[25]

The live birth rates in our trial group, 71.6% and 70.9% respectively for the LMWH and standard care arm are comparable to the live birth rates in pregnant women with unexplained recurrent miscarriage in cohort studies,[26] the original ALIFE trial (62% aspirin, 67% placebo. 69% for aspirin plus LMWH)[19] and in women with recurrent miscarriage and positive for thyroid peroxidase antibodies (TPO-ab) (70% thyroxine, 69% placebo)[27] (68% thyroxine, 62% placebo).[28]

Based on our findings, we do not advise use of LMWH in women with recurrent pregnancy loss and confirmed inherited thrombophilia. Extrapolating our findings, we also advise against screening for inherited thrombophilia in women with recurrent pregnancy loss. Although some patients and physicians may value knowing about a factor that is associated with recurrent pregnancy loss, this association was recently challenged. A systematic review of the prevalence of thrombophilia in women with recurrent miscarriage found it to be the same as that of the general population and therefore suggested that LMWH would not prevent recurrent pregnancy loss.[29]

This trial will have a significant impact on international guidelines and clinical care of women with recurrent miscarriage. Although safe, daily subcutaneous injections are burdensome for women, costly, and should be avoided if not beneficial. The ALIFE2 trial has answered the long-standing debate as to whether screening women for inherited thrombophilia and treating the positives with LMWH prevents early miscarriage. This will save health care services, e.g., the NHS a significant amount of money. An inherited thrombophilia test panel costs £400 per patient and approximately 50,000 women have recurrent miscarriage in the UK each year this trial has the potential to save £20 million per year. The costs of thrombophilia screening in the USA are considerably higher, $1,256 per test panel.

It is notable that 28% of women in our trial lost badly wanted pregnancies; these unexplained pregnancy losses will be the focus of further research so that investigators can now search for other modifiable factors to prevent early pregnancy loss.

In conclusion, our trial shows that LMWH does not increase live birth rates in women with recurrent pregnancy loss and confirmed inherited thrombophilia compared with standard surveillance.

Research in context

Evidence before this study

Prior to commencing the study, the co-authors undertook a Cochrane review of aspirin and/or heparin for women with unexplained recurrent pregnancy loss (RPL) with or without inherited thrombophilia. In women with inherited thrombophilia and RPL this Cochrane meta-analysis compared, low molecular weight heparin (LMWH) to aspirin finding a (risk ratio (RR) of live birth 1.21, 95% CI 0.79 to 1.87) and LMWH and aspirin to no treatment finding a (RR of live birth 1.25, 95% CI 0.74 to 2.12). This Cochrane review concluded that the studies including women with inherited thrombophilia were underpowered and that randomised controlled trials focusing on women with RPL and inherited thrombophilia, only are urgently needed.

Added value of this study

Currently, many women with recurrent miscarriage across the world are tested for inherited thrombophilia and if confirmed positive treated with daily subcutaneous LMWH, despite the absence of evidence that such treatment is beneficial. The ALIFE2 trial fills the current evidence gap that has been put forward by the Cochrane review and international guidelines which express uncertainty as to whether testing for inherited thrombophilia is warranted in RPL and whether LMWH prevents subsequent miscarriage. The ALIFE2 trial has demonstrated that the live birth rate in both study arms is not different, whereas the risk of side effects is increased in the LMWH group. Despite recruiting women from 39 hospitals, from 5 countries, it took over 8 years to randomise sufficient participants to this study. Hence, we suggest that this is the definitive trial on this topic.

Implications of all the available evidence

This finding decreases the treatment burden for women who do not have to self-administer daily injections throughout pregnancy, and will save health services significant costs, such as expensive inherited thrombophilia tests for women with recurrent pregnancy loss as well as costs for LMWH in those who are tested positive.

The results mean that researchers can focus research on other solutions for RPL after decades of investigating anticoagulants.

Contributors

SM and MG designed the original trial protocol which was revised when UK joined (SQ). SM and SQ applied for the research grants. MG, SM, SQ and Warwick clinical trials unit coordinated the trial. KB and LH accessed and verified the data and did the statistical analysis. SQ, SM and MG interpreted the data and wrote the manuscript. All authors revised the manuscript and approved the final submitted version.

Declaration of interests

MG received research and educational grants from Guerbet, Merck, and Ferring, not related to the presented work, paid to their institution. SM received consulting fees from, Bayer, Pfizer, Boehringer-Ingelheim, Portola-Alexion, Abbvie, BMS Pfizer, Norgine, Viatris, Sanofi, GSK, Aspen not related to the presented work, paid to their institution. FB received research grants from and is a member of the advisory board of Merck B.V. and a lecture fee from Besins Healthcare,

Data sharing

The study protocols will be made available with publication. The deidentified participant data can be requested by contacting the corresponding author after approval of a proposal and with a signed data access agreement

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