Heparin for women with recurrent miscarriage and inherited thrombophilia (ALIFE2) an international open-label, randomised controlled trial

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See Comment page 6

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Summary

Background Anticoagulant therapy might reduce the number of miscarriages and adverse pregnancy outcomes in women with recurrent pregnancy loss and inherited thrombophilia. We aimed to assess use of low-molecular-weight heparin (LMWH) versus standard care in this population.

Methods The ALIFE2 trial was an international open-label, randomised controlled trial undertaken in hospitals in the UK (n=26), the Netherlands (n=10), the USA (n=2), Belgium (n=1), and Slovenia (n=1). Women aged 18–42 years who had two or more pregnancy losses and confirmed inherited thrombophilia, and who were trying to conceive or were already pregnant (≤7 weeks' gestation), were eligible for inclusion. Women were randomly assigned (1:1) to use low-dose LMWH or not (alongside standard care in both groups) once they had a positive urine pregnancy test. LMWH was started at or before 7 weeks' gestation and continued until the end of pregnancy. The primary outcome measure was livebirth rate, assessed in all women with available data. Safety outcomes included bleeding episodes, thrombocytopenia, and skin reactions, and were assessed in all randomly assigned women who reported a safety event. The trial was registered within the Dutch Trial Register (NTR3361) and EudraCT (UK: 2015-002357-35).

Findings Between Aug 1, 2012, and Jan 30, 2021, 10 625 women were assessed for eligibility, 428 were registered, and 326 conceived and were randomly assigned (164 to LMWH and 162 to standard care). 116 (72%) of 162 women with primary outcome data in the LMWH group and 112 (71%) of 158 in the standard care group had livebirths (adjusted odds ratio 1·08, 95% CI 0·65 to 1·78; absolute risk difference, 0·7%, 95% CI –9·2% to 10·6%). 39 (24%) of 164 women in the LMWH group and 37 (23%) of 162 women in the standard care group reported adverse events.

Interpretation LMWH did not result in higher livebirth rates in women who had two or more pregnancy losses and confirmed inherited thrombophilia. We do not advise use of LMWH in women with recurrent pregnancy loss and inherited thrombophilia, and we advise against screening for inherited thrombophilia in women with recurrent pregnancy loss.

Funding National Institute for Health and Care Research and the Netherlands Organization for Health Research and Development.

Introduction

Recurrent miscarriage, defined as the loss of two or more pregnancies, affects about 3% of couples trying to conceive. Experiencing recurrent miscarriage can have a profound impact on physical and psychological wellbeing.[1,2] Thrombophilia has been implicated in the cause of recurrent miscarriage, partly 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 about 15% of women with recurrent miscarriage.[4–11] However, although inherited thrombophilia such as factor V Leiden, prothrombin G20210A 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 formal guidance, many clinicians prescribe heparin to women with recurrent miscarriage and inherited thrombophilia.[16] The European Recurrent Pregnancy Loss guidelines recommended "research into the effect of anticoagulant treatment for women with recurrent pregnancy loss and hereditary thrombophilia",[4,5] something that was echoed in UK guidelines and a multidisciplinary 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 livebirth rates, as compared to standard care.

Research in context

Evidence before this study

Beofre commencing the study, the coauthors undertook a Cochrane review of aspirin, heparin, or both for women with unexplained recurrent pregnancy loss with or without inherited thrombophilia. In women with inherited thrombophilia and recurrent pregnancy loss, comparison of treatment with low-molecular-weight heparin (LMWH) versus aspirin gave a risk ratio (RR) of livebirth of 1·21 (95% CI 0·79–1·87) and comparison of LMWH plus aspirin with no treatment gave an RR of livebirth of 1·25 (95% CI 0·74–2·12). This Cochrane review concluded that the studies including women with inherited thrombophilia were underpowered, and that randomised controlled trials focusing on women with recurrent pregnancy loss and inherited thrombophilia only were 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 highlighted by both the Cochrane review and international guidelines, which express uncertainty as to whether testing for inherited thrombophilia is warranted in recurrent pregnancy loss, and whether LMWH prevents subsequent miscarriage. The ALIFE2 trial has demonstrated that the livebirth rate in both study arms is not different, whereas the risk of side-effects is increased in the LMWH group. Despite recruiting women from 40 hospitals, from five countries, it took more than 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 costs, such as expensive inherited thrombophilia tests for women with recurrent pregnancy loss, as well as costs for LMWH in those who test positive. The results also mean that researchers can focus research on other solutions for recurrent pregnancy loss after decades of investigating anticoagulants.

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See **Online** for appendix

Methods

Study design and participants

The ALIFE2 study was an international, multicentre, 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: the Amsterdam University Medical Centers in the Netherlands, and the 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 the National Research Ethics Service, the Medicines and Healthcare products Regulatory Agency, and the Health Research Authority. Written informed consent was obtained from all participants prior to randomisation.

Women aged 18–42 years at time of randomisation were eligible if they had recurrent miscarriage (≥2 consecutive miscarriages, 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 bodyweight lower than 50 kg, an indication for anticoagulant treatment during pregnancy as assessed by the treating physician, contraindications to LMWH, known allergy to at least three different LMWH preparations, and previous inclusion in the ALIFE2 study.

Randomisation and masking

Women who were eligible for the study were recruited in recurrent miscarriage or vascular medicine or haematology clinics prior to pregnancy or before 7+0 weeks' gestation. Women were informed about the study prior to pregnancy. Women were instructed to undergo a urine pregnancy test as soon as their menstrual periods were delayed, or a pregnancy was suspected. In the UK, we aimed to enrol women into the study before pregnancy. Participants then contacted research teams as soon as they were pregnant. If the hospital pregnancy test was positive, individuals were randomly allocated. 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 randomly allocated 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 six, stratified for maternal age (<36 years 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 years or ≥36 years), and number of prior miscarriages (2 or ≥3). There was concealment of allocation sequence 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.

Procedures

LMWH consisted of prefilled syringes containing enoxaparin 40 mg (Clexane [Sanofi-Aventis, Paris, France] or Inhixa [Techdow Pharma, Guildford, UK]), dalteparin 5000 IU (Fragmin [Pfizer, Sandwich, UK]), tinzaparin 4500 IU (Innohep [Leo Pharma, Ballerup, Denmark]), or nadroparin 3800 IU (Fraxiparin, [GlaxoSmithKline, London, UK]); doses were not adjusted to bodyweight. 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 It was continued throughout pregnancy. Women were instructed to discontinue LMWH when labour started. Individuals allocated to LMWH were discouraged from using antithrombotic medications, or other medications that affect haemostasis, including non-steroidal anti-inflammatory drugs. Low-dose aspirin (≤150 mg daily) to decrease the risk for pre-eclampsia 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 400 µg 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 bleeding, haematuria, skin reactions at the injection sites, and gastrointestinal complaints were recorded during every contact.

Outcomes

The primary outcome measure was livebirth after 24+0 weeks' gestation. Secondary outcomes were incidence of and type of miscarriage (biochemical, first trimester, or second trimester), ectopic pregnancy, termination of pregnancy, and obstetric complications including deep vein thrombosis, pulmonary embolism, pre-eclampsia, haemolysis, elevated liver enzymes, and low platelets (also known as HELLP syndrome), small for gestational age (defined as birthweight <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

The study hypothesis was that LMWH would increase the rate of livebirth as compared with no LMWH. In the first ALIFE study, which included women with unexplained recurrent miscarriage, the occurrence of livebirth in the subgroup of women with inherited thrombophilia and who became pregnant was 60% in those who were randomised to placebo.[19] Assuming a livebirth 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 among health-care providers and participants.[18] The UK team aimed to recruit women before they conceived, 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 dropout due to non-compliance, loss to follow-up, and exclusion from the study (eg, ectopic pregnancy). This recruitment target was estimated to deliver the 324-participant randomisation requirement.

Primary and secondary outcome analyses included all available data from all women who were randomised and did not withdraw consent to be followed up. Safety outcomes were assessed in all women who reported safety events. The primary outcome of livebirth after 24+0 weeks' gestation was compared across randomised treatment groups using a χ^2 test with continuity correction, and then sensitivity analysis was undertaken using logistic regression to adjust for stratification factors. Absolute risk differences with 95% CI were also calculated.[20] Prespecified exploratory subgroup analyses were performed to investigate the treatment effect within the factors of age group (<36 years *vs* ≥36 years), number of previous miscarriages (2 *vs* ≥3), previous livebirth (yes *vs* 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.

The trial was registered within the Dutch Trial Register (NTR3361) and EudraCT (UK: 2015-002357-35).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Enrolment took place between Aug 1, 2012, and Jan 30, 2021, with a pause to recruitment due to the COVID-19 pandemic between March 24 and May 18, 2020. 428 women were registered and 326 women were randomly allocated (figure 1, table 1). The trial was stopped when the planned recruitment target was reached. In the UK, 10 625 women with recurrent miscarriage were assessed for eligibility, with the most common reason for ineligibility being not having an inherited thrombophilia (89·6%; figure 1). Figures for screening were not collected in sites managed by the Netherlands, and participants were randomly allocated once pregnant. 164 women were allocated to LMWH plus standard care, and 162 women to standard care alone. In the standard care group 30 participants received LMWH, of whom 18 started heparin treatment before 12 weeks' of gestation. The other 12 participants received LMWH after 12 weeks' of gestation, with six of those 12 participants starting after 28 weeks' of gestation, as they were assessed as needing LMWH for thromboprophylaxis as per Royal College of Obstetricians and Gynaecologists guidelines.[21]

The mean age of the participants was 33 years (SD 5·3), with about a third being older than 36 years, and most were White (table 1). The median number of miscarriages prior to randomisation was 3 (IQR 2–4), and two-thirds of women had a history of three or more miscarriages. The most common thrombophilia types were heterozygosity for factor V Leiden, prothrombin G20210A mutation, and protein S deficiency (table 1). Aspirin was used as co-medication in 36 (11%) of 326 women.

Details about type of LMWH were available for 157 (96%) of the 164 women randomly allocated to receive it. The most commonly administered LMWH was enoxaparin (114 women [73%]), followed by dalteparin (28 [18%]), tinzaparin (13 [8%]), and nadroparin (2 [1%]). Four participants had the type of LMWH changed during the trial: two participants from enoxaparin to dalteparin, and two participants from dalteparin to nadroparin.

Of the 326 randomised participants, 320 (98%) had primary outcome data available (figure 1, table 2). Livebirth rates were 116 (72%) of 162 in the LMWH group and 112 (71%) of 158 in the standard care group. No significant difference was detected between groups with either the unadjusted analysis (odds ratio [OR] 1·04 [95% CI 0·64 to 1·68], p=0·99) or adjusted analysis (1·08 [0·65 to 1·78], p=0·77). The absolute risk difference between groups was 0·7% (95% CI –9·2 to 10·6).

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

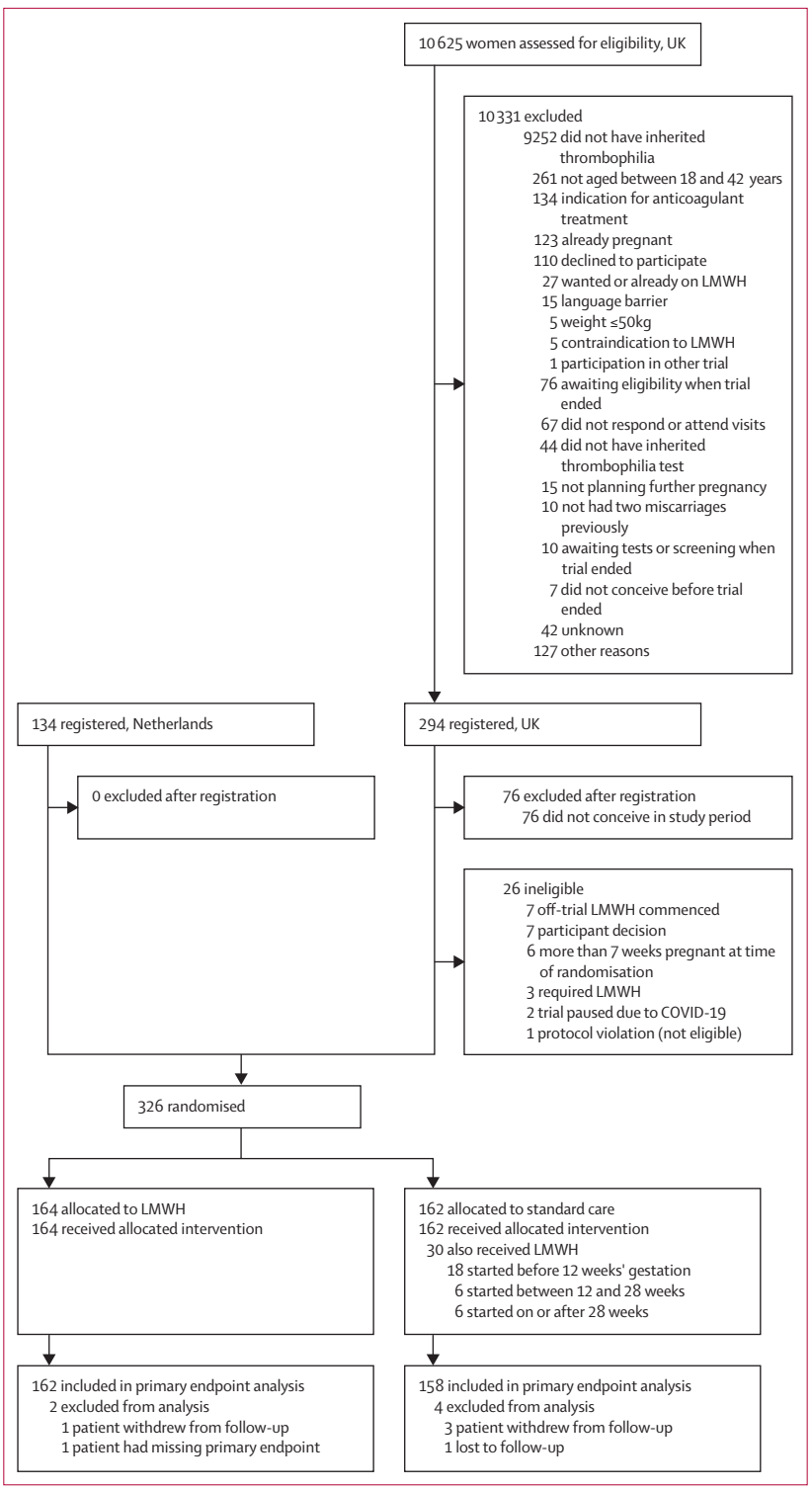

Figure 1: **Trial profile**
LMWH=low-molecular-weight heparin.

	LMWH (n=164)	Standard care (n=162)
Age (years)*		
<36	105 (64%)	103 (64%)
≥36	59 (36%)	59 (36%)
Mean (SD)	33·5 (5·2)	33·3 (5·3)
Median (IQR)	34 (30–38)	33 (29–38)
Number of miscarriages*		
2	46 (28%)	52 (32%)
≥3	118 (72%)	110 (68%)
Number of miscarriages (pre-randomisation)		
Mean (SD)	3·2 (1·2)	3·1 (1·2)
Median (IQR)	3 (2–4)	3 (2–4)
Tertiary centre*		
Yes	143 (87%)	139 (86%)
No	21 (13%)	23 (14%)
Randomising study team*		
UK	96 (59%)	96 (59%)
Netherlands	68 (41%)	66 (41%)
BMI (kg/m²)		
Median (IQR)	24·4 (22·5–27·7)	24·3 (22·1–27·5)
Ethnicity		
White	133 (81%)	136 (84%)
Mixed race	3 (2%)	1 (1%)
Southeast Asian	7 (4%)	9 (6%)
Other Asian	4 (2%)	2 (1%)
Black Caribbean	1 (1%)	1 (1%)
Black African	9 (5%)	7 (4%)
Other	5 (3%)	5 (3%)
Missing	2 (1%)	1 (1%)
Current smoker		
Yes	16 (10%)	15 (9%)
No	135 (82%)	140 (86%)
Missing	13 (8%)	7 (4%)
Co-medication†		
Folic acid	135 (82%)	121 (75%)
Aspirin	16 (10%)	20 (12%)
Progesterone	24 (15%)	19 (12%)
Type of thrombophilia		
Factor V Leiden mutation; heterozygous	95 (58%)	89 (55%)
Factor V Leiden mutation; homozygous	5 (3%)	0 (0%)
Prothrombin G20210A mutation; heterozygous	39 (24%)	44 (27%)
Prothrombin G20210A mutation; homozygous	0	2 (1%)
Antithrombin deficiency	2 (1%)	5 (3%)
Protein C deficiency	5 (3%)	8 (5%)
Protein S deficiency	23 (14%)	21 (13%)
Combined thrombophilia‡	5 (3%)	7 (4%)
(Continued from previous column)		
Family history		
Reported DVT or PE	19 (12%)	17 (10%)
Reported miscarriage	55 (34%)	62 (38%)
Parental chromosome abnormality		
Yes	1 (1%)	1 (1%)
No	112 (68%)	117 (72%)
Not tested	51 (31%)	44 (27%)
Confirmed thyroid autoimmunity		
Yes	5 (3%)	6 (4%)
No	137 (84%)	140 (86%)
Not known	22 (13%)	16 (10%)
Abnormal thyroid stimulating hormone§		
Yes	7 (4%)	5 (3%)
No	142 (87%)	146 (90%)
Not known	15 (9%)	11 (7%)
Septate uterus reported¶		
Yes	0	2 (1%)
Conception		
Spontaneous	143 (87%)	139 (86%)
Intrauterine insemination	2 (1%)	7 (4%)
Donor insemination	0	3 (2%)
Ovulation induction	5 (3%)	4 (2%)
IVF or ICSI	14 (9%)	8 (5%)
Missing	0	1 (1%)

Data are n (%) unless stated otherwise. DVT=deep vein thrombosis. ICSI=intracytoplasmic sperm injection. IVF=in vitro fertilisation. LMWH=low-molecular-weight heparin. PE=pulmonary embolism. *Stratification variables. †Multiple co-medications are recorded for some patients. Progesterone reported was started in luteal phase or early pregnancy. An additional two participants were given progesterone at 24 weeks' gestation or later but have not been reported. ‡Some patients had more than one thrombophilia. §Treated as per local guidelines. ¶Uterine malformations were not screened for in all patients and in all sites.

Table 1: **Baseline characteristics**

The results of the planned subgroup analyses of livebirth rates revealed no evidence of efficacy of LMWH in any of the prespecified subgroups (with all 95% CIs including 1), and no significant interaction effect between these subgroups, with all 95% CIs overlapping (figure 2). A post-hoc exploratory analysis was undertaken comparing women randomised to and receiving LMWH with women who were randomised to standard care and who didn't receive LMWH within the first 12 weeks, and similar results were found. Livebirths occurred in 116 (72%) of 162 women in the LMWH group, 112 (71%) of 158 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 livebirth 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, such as premature delivery and small for gestational age. As expected, low-dose LMWH in pregnancy appeared to be safe; there was no increase in minor bleeding or major bleeding in those randomised to LMWH as compared with 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 clinical 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 six 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 we 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 UK and the Netherlands, with some women recruited from Belgium, Slovenia, and the USA. Compliance with allocated treatment was good. While 30 women in the standard care group took LMWH at some point in pregnancy, only 18 women were in the first trimester when LMWH use might 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] It is important to note that 70% of our study population had three or more miscarriages, and that there was no significant interaction between treatment assignment and number of miscarriages (2 *vs* ≥3) with respect to livebirth 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 arising from waiting for ultrasound scanning.

	LMWH (n=162)	Standard care (n=158)	Unadjusted analysis*	Adjusted analysis†	Absolute risk difference
Livebirth	116 (72%)	112 (71%)	1·04 (0·64 to 1·68); p=0·99	1·08 (0·65 to 1·78); p=0·77	0·7% (95% CI −9·2% to 10·6%)
Pregnancy loss	46 (28%)	46‡ (29%)	..	..	..

Data are n (%) unless stated otherwise. LMWH=low-molecular-weight heparin. *Odds ratio (95% CI) and χ^2 test p value with continuity correction. †Odds ratio (95% CI) and p value logistic regression adjusted for maternal age (<36 years, ≥36 years), number of miscarriages (2, ≥3), tertiary or non-tertiary centre, and randomising country (UK, Netherlands), with the standard surveillance group as the reference group. ‡One set of triplets in the standard care group was counted as a livebirth as two of the three fetuses were livebirths; the other was terminated at 8 + 3 weeks' gestation. This termination has not been counted in this table.

Table 2: **Pregnancy outcome (livebirth rates)**

	LMWH	Standard care
Type of early pregnancy loss		
Biochemical loss	7/162 (4%)	6/158 (4%)
Ectopic pregnancy	3/162 (2%)	1/158 (1%)
Intrauterine pregnancy identified on ultrasound scan		
Miscarriage <12 weeks	34/152* (22%)	34/151* (23%)
Termination of pregnancy	1/152 (1%)	2/151 (1%)
Reached second trimester		
Miscarriage 12–24 weeks	1/117 (1%)	3/115 (3%)
Reached third trimester (livebirth)		
Stillbirth	0/116	0/112
Total number of pregnancy losses	46/162 (28%)	46/158 (29%)
Obstetric complications in third trimester	n=116	n=112
Pre-eclampsia or HELLP syndrome	4/114 (4%)	3/109 (3%)
Small for gestational age†	12/111 (11%)‡	15/104 (14%)
Placental abruption	1/114 (1%)	1/109 (1%)
Premature birth <37 weeks' gestation	15/116 (13%)	15/112 (13%)
Congenital anomalies	3/104 (3%)§	2/103 (2%)¶
Other complications during entire pregnancy	n=164	n=162
Confirmed deep vein thrombosis or pulmonary embolism	1/154 (1%)	0/144 (0%)
Easy bruising	73/164 (45%)	16/162 (10%)
Adverse events during entire pregnancy	n=164	n=162
Post-partum bleeding >500 mL	7 events	12 events
Major bleeding >100 mL	3 events	3 events
Clinically relevant non-major bleeding	9 events	14 events
Minor bleeding	35 events	27 events
Heparin-induced thrombocytopenia	0 events	0 events
Skin reactions at injection site	3 events	1 events‖
Total number of adverse events	57	57
Total number of participants reporting an adverse event	39/164 (24%)	37/162 (23%)

IUGR=intrauterine growth restriction. LMWH=low-molecular-weight heparin. *A set of twins was miscarried (counted as one pregnancy); this happened once in both arms. †Small for gestational age was calculated using the customised GROW chart, less than the tenth centile. ‡One participant had twins who both had IUGR; this has been counted as one event. §One baby had respiratory distress, stenosis anus with fistula, and anorectal malfunction surgery to correct imperforate anus. One baby had an extra toe on the right foot. One baby had an undescended right testis. ¶One baby had oesophageal atresia and one baby had Down syndrome. ‖Took LMWH although randomised to standard care.

Table 3: **Pregnancy loss and secondary outcome measures**

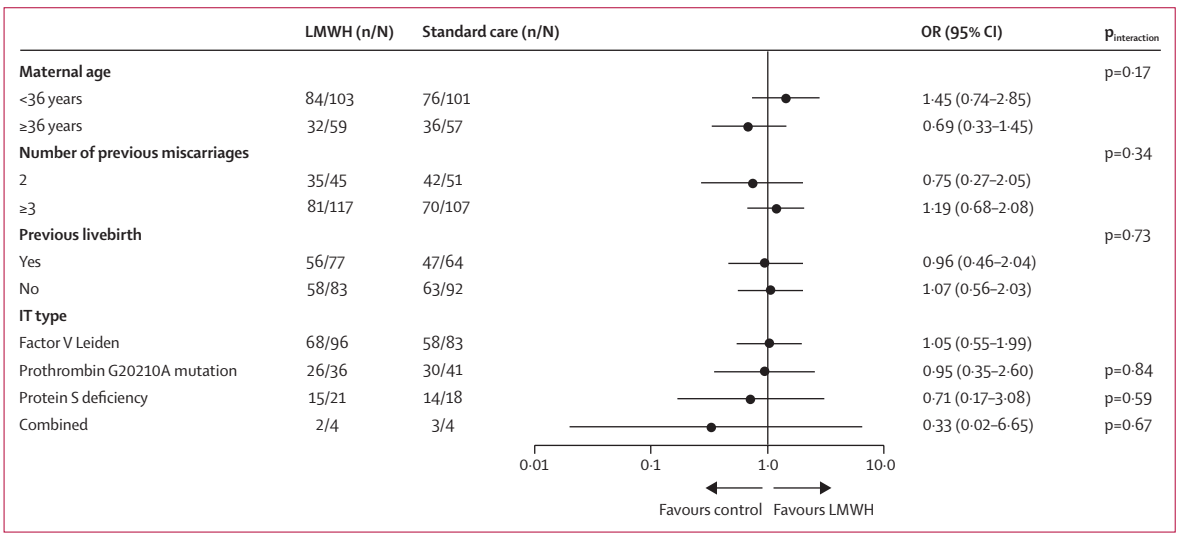

Figure 2: **Forest plot of subgroup analyses**
IT=inherited thrombophilia. LMWH=low-molecular-weight heparin. OR=odds ratio.

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, this 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 livebirth 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 livebirth rate is unlikely. Finally, the lack of masking of participants, treating physicians, and outcome assessors might be a limitation. However, the primary outcome event of livebirth is unlikely to be subject to diagnostic suspicion bias.

Our results answer a question posed in the literature, previously 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 was associated with an increased chance of livebirth (OR 10·6, 95% CI 5·0–22·3), concluding that LMWH was beneficial in preventing pregnancy loss.[24] Other authors suggested that LMWH would not improve livebirth rates in women with pregnancy loss and thrombophilia. A meta-analysis of mainly subgroups of eight small trials, comparing LMWH with no LMWH during pregnancy in women with inherited thrombophilia and heterogeneous pregnancy morbidity, did not demonstrate a clinical benefit of LMWH (relative risk 0·81; 95% CI 0·55–1·19)[25] but agreed the ALIFE2 results were needed.

The livebirth rates in our trial group, 72% for the LMWH group and 71% for the standard care group, are comparable with the livebirth rates in pregnant women with unexplained recurrent miscarriage in cohort studies,[26] the original ALIFE trial (62% aspirin, 67% placebo, and 69% for aspirin plus LMWH),[19] and in women with recurrent miscarriage and positive for thyroid peroxidase antibodies in two studies (70% thyroxine and 69% placebo,[27] and 68% thyroxine and 62% placebo[28]).

Based on our findings, we advise against the 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 provided an answer to the long-standing debate as to whether screening women for inherited thrombophilia and treating the positive individuals with LMWH prevents early miscarriage. This will save health-care services such as the UK National Health Service a significant amount of money. As 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, at US$1256 per test panel.

Notably, 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.

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Contributors
SM and MG designed the original trial protocol, which was revised with SQ when the UK joined. 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, and Aspen not related to the presented work, paid to their institution. All other authors declare no competing interests.

Data sharing
The UK and Dutch protocols are available online. The de-identified 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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For the **study protocols** see https://warwick.ac.uk/fac/sci/med/research/ctu/trials/alife2/ and https://zorgevaluatienederland.nl/evaluations/alife-2?activeTab=tab-information