ORIGINAL ARTICLE

Aspirin plus Heparin or Aspirin Alone in Women with Recurrent Miscarriage

Stef P. Kaandorp, M.D., Mariëtte Goddijn, M.D., Ph.D., Joris A.M. van der Post, M.D., Ph.D., Barbara A. Hutten, Ph.D., Harold R. Verhoeve, M.D., Karly Hamulyák, M.D., Ph.D., Ben Willem Mol, M.D., Ph.D., Nienke Folkeringa, M.D., Ph.D., Marleen Nahuis, M.D., Dimitri N.M. Papatsonis, M.D., Ph.D., Harry R. Büller, M.D., Ph.D., Fulco van der Veen, M.D., Ph.D., and Saskia Middeldorp, M.D., Ph.D.

ABSTRACT

BACKGROUND

Aspirin and low-molecular-weight heparin are prescribed for women with unexplained recurrent miscarriage, with the goal of improving the rate of live births, but limited data from randomized, controlled trials are available to support the use of these drugs.

METHODS

In this randomized trial, we enrolled 364 women between the ages of 18 and 42 years who had a history of unexplained recurrent miscarriage and were attempting to conceive or were less than 6 weeks pregnant. We then randomly assigned them to receive daily 80 mg of aspirin plus open-label subcutaneous nadroparin (at a dose of 2850 IU, starting as soon as a viable pregnancy was demonstrated), 80 mg of aspirin alone, or placebo. The primary outcome measure was the live-birth rate. Secondary outcomes included rates of miscarriage, obstetrical complications, and maternal and fetal adverse events.

RESULTS

Live-birth rates did not differ significantly among the three study groups. The proportions of women who gave birth to a live infant were 54.5% in the group receiving aspirin plus nadroparin (combination-therapy group), 50.8% in the aspirin-only group, and 57.0% in the placebo group (absolute difference in live-birth rate: combination therapy vs. placebo, –2.6 percentage points; 95% confidence interval [CI], –15.0 to 9.9; aspirin only vs. placebo, –6.2 percentage points; 95% CI, –18.8 to 6.4). Among 299 women who became pregnant, the live-birth rates were 69.1% in the combination-therapy group, 61.6% in the aspirin-only group, and 67.0% in the placebo group (absolute difference in live-birth rate: combination therapy vs. placebo, 2.1 percentage points; 95% CI, –10.8 to 15.0; aspirin alone vs. placebo –5.4 percentage points; 95% CI, –18.6 to 7.8). An increased tendency to bruise and swelling or itching at the injection site occurred significantly more frequently in the combination-therapy group than in the other two study groups.

CONCLUSIONS

Neither aspirin combined with nadroparin nor aspirin alone improved the live-birth rate, as compared with placebo, among women with unexplained recurrent miscarriage. (Current Controlled Trials number, ISRCTN58496168.)

From the Department of Obstetrics and Gynecology (S.P.K., J.A.M.P.), the Center for Reproductive Medicine (M.G., F.V.), the Department of Clinical Epidemiology, Biostatistics, and Bioinformatics (B.A.H.), and the Department of Vascular Medicine (H.R.B), Academic Medical Center, University of Amsterdam, Amsterdam; Onze Lieve Vrouwe Gasthuis, Amsterdam (H.R.V.); University Hospital Maastricht, Maastricht University, Maastricht (K.H.); Máxima Medical Center, Veldhoven (B.W.M.); University Medical Center Groningen, University of Groningen, Groningen (N.F.); Medisch Spectrum Twente Hospital Group, Enschede (M.N.); Amphia Hospital, Breda (D.N.M.P.); and Leiden University Medical Center, University of Leiden, Leiden (S.M.) — all in the Netherlands. Address reprint requests to Dr. Goddijn at the Center for Reproductive Medicine, H4-205, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, the Netherlands, or at m.goddijn@amc.uva.nl.

This article (10.1056/NEJMoa1000641) was published on March 24, 2010 at NEJM.org.

N Engl J Med 2010.

Approximately 1% of all women trying to conceive have recurrent miscarriage, defined as three previous miscarriages; when recurrent miscarriage is defined as two previous miscarriages, the proportion rises to 5%.[1] In half of such patients, no underlying cause of miscarriage can be identified.[2,3] Although various interventions have been suggested to improve rates of live birth in such cases, no effective treatment has been identified.

It has been suggested that in women with recurrent miscarriage and a diagnosis of the antiphospholipid syndrome, treatment with aspirin and heparin may improve the pregnancy outcome, although findings from available randomized trials have been inconsistent.[4-6] On the basis of presumed similarities in pathogenesis between recurrent miscarriage associated with the antiphospholipid syndrome and unexplained recurrent miscarriage,[7] aspirin and low-molecular-weight heparin are frequently prescribed for women with unexplained recurrent miscarriage or those with recurrent miscarriage and inherited thrombophilias. However, limited data from randomized, controlled trials are available to support this approach.[8-11] In this multicenter, randomized, placebo-controlled trial, called the Anticoagulants for Living Fetuses (ALIFE) study, we investigated whether aspirin combined with low-molecular-weight heparin or aspirin alone, as compared with placebo, would improve the live-birth rate among women with unexplained recurrent miscarriage.

Methods

Study Population

From February 2004 through January 2008, we evaluated patients at three university hospitals and five teaching hospitals in the Netherlands. Women between the ages of 18 and 42 years were eligible if they had had unexplained recurrent miscarriage and were attempting to conceive or were pregnant, with a gestational age of less than 6 weeks. Previous miscarriage was defined as pregnancy loss at a gestational age of 20 weeks or less. The definition of miscarriage included documentation of pregnancy by a positive pregnancy test and clinical manifestations of miscarriage (e.g., abdominal pain, cramps, and vaginal bleeding); it did not include the loss of a biochemical pregnancy. Recurrent miscarriage was defined as at least two miscarriages. Unexplained recurrent miscarriage was diagnosed in cases of normal karyotypes of both partners, the absence of uterine disease on pelvic ultrasonography, the absence of the antiphospholipid syndrome (on the basis of testing for lupus anticoagulant and anticardiolipin IgG and IgM antibodies), and a normal fasting level of homocysteine (<16 μmol per liter). Women who had previous venous or arterial thromboembolism, an indication for anticoagulant treatment during pregnancy, or endocrine disorders (e.g., diabetes mellitus or untreated thyroid dysfunction) were excluded from the study.

Participating women were tested once for factor V Leiden and the prothrombin G20210A mutation and plasma activity levels of protein C, protein S, and antithrombin. Deficiencies were defined as less than 70% of normal activity for protein C, less than 65% of normal activity for total protein S, and less than 80% of normal activity for antithrombin.

The study protocol was approved by the institutional review board at each center. All patients provided written informed consent.

Study Design and Treatment Regimen

Women were randomly assigned to receive aspirin combined with low-molecular-weight heparin (combination-therapy group), aspirin alone, or placebo either before conception or at a gestational age of less than 6 weeks. Randomization was performed centrally by a computer program with minimization for maternal age (<36 or ≥36 years) and the number of miscarriages (2 or ≥3), stratified according to study center.

Aspirin in the form of calcium carbasalate (Ascal, Meda Pharma) was administered at a daily dose of 100 mg, which is equivalent to 80 mg of acetylsalicylic acid. Aspirin or placebo was started at the time of randomization and continued until a gestational age of 36 weeks or was stopped at the time of miscarriage, a diagnosis of ectopic pregnancy, or premature delivery. Aspirin and placebo were packed in sachets of identical appearance; patients, doctors, and trial nurses were all unaware of the study-group assignments.

Open-label low-molecular-weight heparin in the form of nadroparin (Fraxiparine, GlaxoSmithKline) was administered subcutaneously at a daily dose of 2850 IU and was initiated when

10.1056/NEJMoa1000641 NEJM.ORG

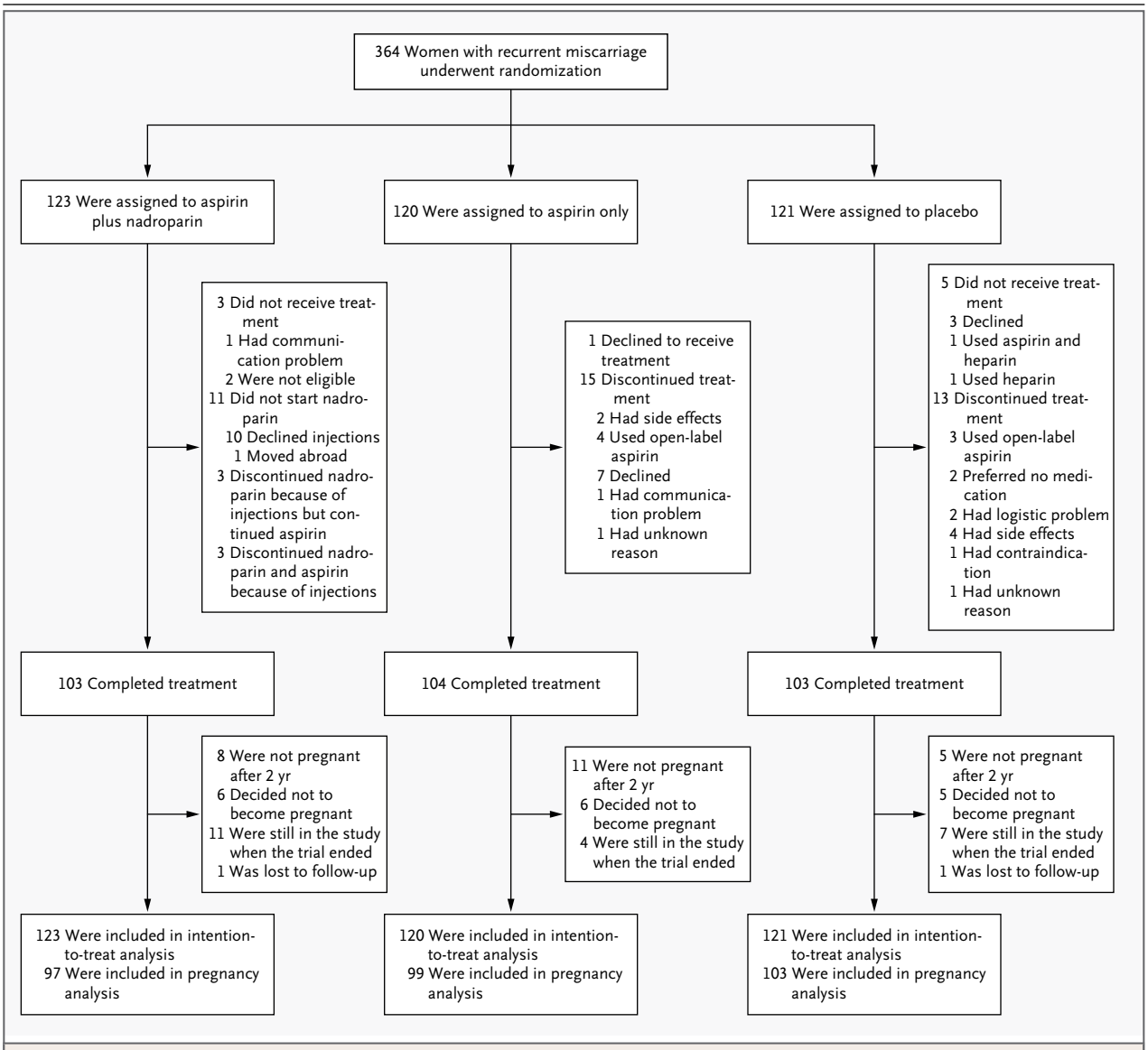

Figure 1. Enrollment and Outcomes.

a viable intrauterine pregnancy was confirmed on ultrasonography, starting at 6 weeks of gestation, and was continued throughout pregnancy; women were instructed to discontinue this medication when labor started. Women were withdrawn from the trial if they had not become pregnant within 2 years after randomization or if they no longer wished to become pregnant; however, these women were included in the intention-to-treat analysis.

All women were advised to take folic acid (400 μg daily), starting before conception and continuing until 10 weeks of gestation, as prophylaxis for neural-tube defects. Women received standard care provided by their own obstetrician throughout pregnancy, including structural fetal ultrasonography at 18 to 22 weeks of gestation. In addition, platelet counts were performed at 12 and 30 weeks of gestation. Women were contacted by telephone by a dedicated research nurse every 3 months throughout the study until completion of the first pregnancy; compliance and side effects were addressed during these calls with the use of a structured form.

Table 1. Baseline Characteristics of the Patients.*

	Aspirin plus Nadroparin (N=123)	Aspirin Only (N=120)	Placebo (N=121)
Age			
Mean — yr	34±5	33±5	34±5
≥36 yr — no. (%)	47 (38.2)	41 (34.2)	44 (36.4)
Body-mass index†	25.4±4.9	25.0±4.8	24.6±4.1
Daily smoking, ≥1 cigarette — no. (%)	23 (18.7)	20 (16.7)	20 (16.5)
Daily alcohol consumption, ≥8 g — no. (%)‡	3 (2.4)	4 (3.3)	9 (7.4)
Dutch nationality — no. (%)	102 (82.9)	102 (85.0)	102 (84.3)
Pregnant at time of randomization — no. (%)	28 (22.8)	33 (27.5)	33 (27.3)
Miscarriage			
Median (range) — no.	3 (2–15)	3 (2–9)	3 (2–12)
≥3 miscarriages — no. (%)	73 (59.3)	71 (59.2)	74 (61.2)
≥1 late miscarriage — no. (%)	40 (32.5)	38 (31.7)	35 (28.9)
Previous live birth — no. (%)	53 (43.1)	45 (37.5)	46 (38.0)
Inherited thrombophilia — no. (%)§			
No. of patients	105	99	98
One or more defects	13 (12.4)	17 (17.2)	17 (17.3)
Factor V Leiden mutation	5 (4.8)	7 (7.1)	9 (9.2)
Prothrombin G20210A mutation	1 (1.0)	3 (3.0)	0
Protein C deficiency	2 (1.9)	1 (1.0)	2 (2.0)
Protein S deficiency	4 (3.8)	5 (5.1)	7 (7.1)
Antithrombin deficiency	2 (1.9)	3 (3.0)	0
Polycystic ovary syndrome — no. (%)	4 (3.3)	3 (2.5)	6 (5.0)

*Plus–minus values are means ±SD.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ P=0.03 for all comparisons.
§ Among patients who were evaluated for inherited thrombophilia, deficiencies were defined as less than 70% of normal activity for protein C, less than 65% for total protein S, and less than 80% for antithrombin.

OUTCOME MEASURES

The primary outcome measure was the rate of live births. Secondary outcomes included rates of miscarriage, intrauterine fetal death (fetal death after 20 weeks of gestation), and obstetrical complications. Such complications included pre-eclampsia, the HELLP syndrome (hemolysis, elevated liver enzymes, and a low platelet count), small size for gestational age (birth weight below the 10th percentile for gestational age and sex), placental abruption, and premature delivery. Premature delivery was classified a priori in three subgroups according to weeks of gestational age (24 to <28, 28 to <32, and 32 to <37).

The rates of maternal thrombocytopenia (defined as a platelet count of <150,000 per cubic millimeter), bleeding episodes (i.e., bleeding from the gums or nose and the amount of vaginal blood loss at delivery), and skin reactions were assessed by telephone at 3-month intervals by the research nurse and verified on the basis of obstetrical medical reports. All infants were examined by an obstetrician. In cases in which a congenital or neonatal abnormality was suspected, a neonatologist made the final diagnosis.

STUDY OVERSIGHT

This study was supported by the Netherlands Organization for Health Research and Development. Aspirin and placebo were packaged and supplied by Meda Pharma. Nadroparin was purchased by subjects from their local pharmacies. The drug's manufacturer, GlaxoSmithKline, provided an unrestricted grant to the investigators.

Table 2. Live-Birth Rate (Primary Outcome).*

Variable	Aspirin plus Nadroparin	Aspirin Only	Placebo	P Value
Intention-to-treat population				
No. of patients	123	120	121	
Live birth — no. (%)	67 (54.5)	61 (50.8)	69 (57.0)	0.63
Relative risk (95% CI)	0.96 (0.76 to 1.19)	0.89 (0.71 to 1.13)	1.00	
Absolute difference in live-birth rate (95% CI) — %	–2.6 (–15.0 to 9.9)	–6.2 (–18.8 to 6.4)	—	
Women who became pregnant				
No. of patients	97	99	103	
Live birth — no. (%)	67 (69.1)	61 (61.6)	69 (67.0)	0.52
Relative risk (95% CI)	1.03 (0.85 to 1.25)	0.92 (0.75 to 1.13)	1.00	
Absolute difference in live-birth rate (95% CI) — %	2.1 (–10.8 to 15.0)	–5.4 (–18.6 to 7.8)	—	

* Absolute differences and relative risks were calculated for the comparison between patients receiving aspirin plus nadroparin (combination-therapy group) and the placebo group and between the aspirin-only group and the placebo group. P values are for all comparisons. CI denotes confidence interval.

None of the sponsors were involved in the preparation of the study protocol, management of the trial, analysis of the data, or preparation of the manuscript. All authors vouch for the accuracy and completeness of the reported data.

STATISTICAL ANALYSIS

We assumed that women who were assigned to receive aspirin alone or placebo had a 75% chance of a live birth.[12] To detect an absolute increase of 15 percentage points in the live-birth rate with the use of combination therapy, we needed to enroll 309 women for a power of 80% at a significance level of 0.05. We aimed to enroll 360 women, since not all women would become pregnant during the trial.[13,14]

The primary outcome was assessed in all women, according to the intention-to-treat principle, as well as in the subgroup of women who conceived. The incidences of preeclampsia, the HELLP syndrome, placental abruption, preterm delivery, small size for gestational age, and congenital or neonatal abnormalities, as well as the estimated amount of vaginal blood loss at delivery, were calculated for women who had an ongoing pregnancy beyond 12 weeks of gestation. Adverse events were evaluated for all women.

Differences in dichotomous outcomes among the three study groups were analyzed with the use of the chi-square test or Fisher's exact test when the expected cell frequencies fell below five. Differences in live-birth rates were expressed as absolute differences and relative risks, with associated 95% confidence intervals, with the placebo group as the reference. One-way analysis-of-variance statistics were calculated to compare continuous outcome measures.

Prespecified analyses included assessments of interactions between study-group assignment and subgroup on the basis of the following potential or established prognostic determinants: the presence or absence of inherited thrombophilia,[15,16] the presence or absence of a previous live birth,[17,18] age (<36 years or ≥36 years),[12,19,20] and the number of miscarriages (2 or ≥3).[12,18,21] Absolute differences in live-birth rates and relative risks for a live birth that were associated with the interventions were calculated for the separate subgroups with the placebo group as the reference. To assess whether the relative risks differed significantly among subgroups, we calculated the difference between the log relative risk and its standard error and used these values to test the interaction.[22] A P value of less than 0.05 was considered to indicate statistical significance.

A data and safety monitoring board, whose members were unaware of the study-group assignments, performed two interim analyses. The first interim analysis was performed after the randomization of 180 women, and a second interim analysis, performed 18 months after study

10.1056/NEJMoa1000641 NEJM.ORG

Table 3. Secondary Outcomes.*

Outcome	Aspirin plus Nadroparin	Aspirin Only	Placebo	P Value†	Absolute Risk Difference (95% CI)	
					Combination Therapy vs. Placebo	Aspirin Only vs. Placebo
Complications of early pregnancy						
No. of patients	123	120	121			
Miscarriage — no. (%)	27 (22.0)	37 (30.8)	31 (25.6)	0.29	−3.7 (−14.3 to 7.0)	5.2 (−6.1 to 16.6)
Ectopic pregnancy — no. (%)	0	1 (0.8)	1 (0.8)	0.55	−0.8 (−2.4 to 0.8)	0.0 (−2.3 to 2.3)
Termination of pregnancy — no. (%)	1 (0.8)	0	1 (0.8)	1.00	0 (−2.3 to 2.3)	−0.8 (−2.4 to 0.8)
Gestational age at miscarriage — wk	8.7±2.3	9.0±2.9	9.1±2.9	0.81	−0.4 (−1.1 to 0.3)	−0.1 (−0.8 to 0.6)
Ongoing pregnancy outcomes						
No. of patients	69	61	70			
Preeclampsia — no. (%)	2 (2.9)	1 (1.6)	1 (1.4)	0.84	1.5 (−3.4 to 6.3)	0.2 (−4.0 to 4.4)
HELLP syndrome — no. (%)	0	1 (1.6)	0	0.31		1.6 (−1.6 to 4.8)
Placental abruption — no. (%)	0	0	0			
Intrauterine fetal death — no. (%)	1 (1.4)	0	1 (1.4)	1.00	0 (−3.9 to 4.0)	−1.4 (−4.2 to 1.4)
Median blood loss at delivery (range) — ml	375 (65 to 1500)	350 (100 to 1800)	400 (50 to 2300)	0.71		
Small for gestational age (<10th percentile) — no. (%)	6 (8.7)	7 (11.5)	5 (7.1)	0.69	1.6 (−7.4 to 10.5)	4.3 (−5.7 to 14.4)
Gestational age at delivery — wk	38.3±3.6	39.5±1.3	39.6±1.6	0.03	−1.3 (−2.2 to −0.4)	−0.1 (−0.6 to 0.4)
Premature delivery — no. (%)						
Any	7 (10.1)	1 (1.6)	3 (4.3)	0.11	5.9 (−2.7 to 14.4)	−2.7 (−8.4 to 3.1)
≥24 to <28 wk	1 (1.4)	0	0			
≥28 to <32 wk	0	0	0			
≥32 to <37 wk	6 (8.7)	1 (1.6)	3 (4.3)			
Multiple gestation — no. (%)	2 (2.9)	0	4 (5.7)	0.38	−2.8 (−9.5 to 3.9)	−5.7 (−11.2 to −0.3)
Maternal adverse events						
No. of patients	123	120	121			
Thrombocytopenia — no. (%)‡	4 (3.3)	0	2 (1.7)	0.17	1.6 (−2.3 to 5.5)	−1.7 (−3.9 to 0.6)
Swelling or itching at injection site — no. (%)	49 (39.8)	0	1 (0.8)	<0.001	39.0 (30.2 to 47.8)	−0.8 (−2.4 to 0.8)
Need to change heparin formulation — no. (%)	0	0	0			
Nosebleed — no. (%)	13 (10.6)	10 (8.3)	11 (9.1)	0.83	1.5 (−6.0 to 9.0)	−0.8 (−7.9 to 6.4)
Bruising — no. (%)	61 (49.6)	23 (19.2)	14 (11.6)	<0.001	38.0 (27.5 to 48.5)	7.6 (−1.5 to 16.7)
Gastrointestinal problem — no. (%)	11 (8.9)	8 (6.7)	11 (9.1)	0.75	−0.1 (−7.3 to 7.0)	−2.4 (−9.2 to 4.4)
Hematuria — no. (%)	1 (0.8)	1 (0.8)	0	0.78	0.8 (−0.8 to 2.4)	0.8 (−0.8 to 2.5)
Bleeding gums — no. (%)	20 (16.3)	15 (12.5)	23 (19.0)	0.38	−2.8 (−12.3 to 6.8)	−6.5 (−15.7 to 2.7)

Neonatal events						
No. of patients	69	61	70			
Congenital or neonatal abnormalities — no. (%)∫	3 (4.3)	5 (8.2)	2 (2.9)	0.39	1.5 (−4.7 to 7.7)	5.3 (−2.6 to 13.3)

* Plus–minus values are means ±SD. Complications of early pregnancy and maternal adverse events were calculated for all 364 women who underwent randomization. Ongoing pregnancy outcomes and neonatal events were evaluated for 200 women with ongoing pregnancy beyond 12 weeks of gestation. HELLP denotes hemolysis, elevated liver enzymes, and low platelet count.
† P values are for all comparisons.
‡ Platelets were measured only in women with ongoing pregnancy beyond 12 weeks of gestation. Thrombocytopenia was defined as a platelet count below 150,000 per cubic millimeter.
∫ In the combination-therapy group, the three events were central adrenal insufficiency; polycystic kidneys in one of trichorial triplets, resulting in death shortly after birth; and medium-chain acyl-coenzyme A dehydrogenase deficiency. In the aspirin-only group, the five events were prenatal supraventricular tachycardia, preauricular tags, and three chromosomal abnormalities (one de novo interstitial deletion 18q21.2, resulting in Pitt–Hopkins syndrome; one trisomy 9 mosaicism, resulting in small size for gestational age and a heart-valve abnormality; and one trisomy 21 [Down's syndrome]). In the placebo group, the two events were multiple structural abnormalities resulting in one fetal twin death and a chromosomal abnormality (trisomy 18) resulting in death 3 days after birth.

recruitment was stopped, included data from 281 women in whom the primary outcome (live birth) had occurred or in whom a miscarriage had occurred by July 1, 2009. On the basis of the second analysis, the board advised discontinuation of the study because of futility. At the time of discontinuation, 22 women were still in follow-up and their medications were stopped. No adjustment was made for the interim analyses of the data.

RESULTS

STUDY POPULATION

A total of 364 women were enrolled, with 123 assigned to the combination-therapy group, 120 to the aspirin-only group, and 121 to the placebo group (Fig. 1). Of these women, 103 (83.7%) in the combination-therapy group, 104 (86.7%) in the aspirin-only group, and 103 (85.1%) in the placebo group received the assigned study intervention. Women who did not start the assigned study intervention were not informed of the study-group assignment and received standard follow-up. The reported reasons for not starting the assigned study intervention are noted in Figure 1. The most frequent reason for discontinuing a study intervention was the occurrence of side effects.

Table 1 summarizes the baseline characteristics of the study population. The mean age of women at randomization was 34 years, and the median number of preceding miscarriages was three. Baseline characteristics were similar across the study groups, except that alcohol use at the time of study entry was more common in the placebo group than in the combination-therapy group or the aspirin-only group.

OUTCOMES

Of the 364 women who underwent randomization, 299 (82.1%) became pregnant, and 197 (54.1% overall and 65.9% of those who became pregnant) had a live birth. Live-birth rates did not differ significantly among the three study groups. The proportions of women who gave birth to a live infant were 54.5% in the combination-therapy group, 50.8% in the aspirin-only group, and 57.0% in the placebo group (absolute difference in live-birth rate: combination therapy vs. placebo, −2.6 percentage points; 95% confidence interval [CI], −15.0 to 9.9; aspirin only vs. placebo, −6.2 percentage points; 95% CI, −18.8

to 6.4) (Table 2). In analyses involving women who became pregnant, live-birth rates also did not differ significantly among the three groups, with rates of 69.1% in the combination-therapy group, 61.6% in the aspirin-only group, and 67.0% in the placebo group (absolute difference in live-birth rate: combination therapy vs. placebo, 2.1 percentage points; 95% CI, –10.8 to 15.0; aspirin only vs. placebo, –5.4 percentage points; 95% CI, –18.6 to 7.8). In a post hoc per-protocol analysis taking into account adherence to the assigned study drug, live-birth rates were very similar to those in the intention-to-treat analysis (for details, see the table in the Supplementary Appendix, available with the full text of this article at NEJM.org).

No significant differences in secondary outcomes were observed among the three groups, except that women in the combination-therapy group delivered approximately 1 week earlier than women in the placebo group (Table 3). An increased tendency to bruise and swelling or itching at the injection site were significantly more common in women in the combination-therapy group than in those in the placebo group. No serious maternal adverse events were reported.

There were no significant interactions between the study-group assignment and the presence or absence of inherited thrombophilia, the presence or absence of a previous live birth, age, or the number of previous miscarriages (Table 4).

DISCUSSION

In this trial, we found that neither aspirin combined with nadroparin nor aspirin alone improved the chance of a live birth in women with a history of unexplained recurrent miscarriage. Live-birth rates were 54.5% in the combination-therapy group, 50.8% in the aspirin-only group, and 57.0% in the placebo group. Among women who became pregnant, these rates were 69.1%, 61.6%, and 67.0%, respectively. Side effects, most notably an increased tendency to bruise and swelling or itching at the injection site, occurred in almost half the women in the combination-therapy group.

The hypothesis that women with unexplained recurrent miscarriage might benefit from aspirin, heparin, or both was based on a presumption that this condition might be caused by thrombosis in decidual vessels.[23,24] In one study, levels of circulating procoagulant microparticles were higher in women with recurrent miscarriage than in control subjects.[25] However, the concept that recurrent miscarriage can be attributed routinely to thrombosis is probably an oversimplification. For example, although activated coagulation factors induce cell death and inhibit the growth of trophoblast cells in thrombomodulin-deficient mice, aspirin or heparin does not reverse defective trophoblast differentiation or the embryonic growth defect.[26] In cultured placental villous tissue obtained from women, both aspirin and heparin were reported to attenuate trophoblast apoptosis.[27]

Some limitations of our study warrant consideration. Our initial sample-size calculation assumed that 309 women would become pregnant and be followed for live birth. We discontinued the trial when 22 women were still in follow-up, on the basis of the judgment by the data and safety monitoring board that further continuation of the study was futile; thus, we do not have complete follow-up data on all enrolled women. However, even in the most extreme case, in which all women in the combination-therapy group would have had a live birth and all women in the placebo group would have had a miscarriage, the absolute live-birth rates would not have differed significantly among the groups. The rate of study-drug adherence was only 85%, a factor that could have increased the statistical uncertainty around the observed absence of effect. Although the point estimates for absolute risk differences suggest that there was no appreciable benefit of aspirin combined with low-molecular-weight heparin or of aspirin alone in women overall or in the subgroup who became pregnant, the 95% confidence interval for the absolute difference in live-birth rate in the latter group indicates that results are compatible with an improvement in the live-birth rate of as much as 15% with aspirin combined with low-molecular-weight heparin, as compared with placebo. A post hoc per-protocol analysis that was limited to the women who became pregnant showed similar point estimates and confidence intervals.

In addition, the use of nadroparin was not blinded. We thought that it would not be feasible to administer subcutaneous placebo injections for a period of 8 months and that the assessment of the primary outcome (i.e., a live birth) was unlikely to be affected by this open-label design.

Table 4. Live-Birth Rate in Prespecified Subgroups.*

Subgroup	Aspirin plus Nadroparin				Aspirin Only				Placebo
	Patients per Subgroup	Absolute Difference in Live-Birth Rate (95% CI)	Relative Risk (95% CI)	P Value for Interaction	Patients per Subgroup	Absolute Difference in Live-Birth Rate (95% CI)	Relative Risk (95% CI)	P Value for Interaction	Patients per Subgroup
	no./total no.				*no./total no.*				*no./total no.*
Inherited thrombophilia†									
Yes	9/13	16.3 (–18.2 to 50.8)	1.31 (0.74 to 2.33)	0.18	11/17	11.8 (–21.1 to 44.6)	1.22 (0.69 to 2.16)	0.32	9/17
No	45/92	–9.1 (–23.9 to 5.7)	0.84 (0.64 to 1.11)		42/82	–6.8 (–22.1 to 8.4)	0.88 (0.67 to 1.17)		47/81
Previous live birth									
Yes	27/53	–7.8 (–27.3 to 11.8)	0.87 (0.61 to 1.24)	0.49	23/45	–7.6 (–28.0 to 12.8)	0.87 (0.60 to 1.27)	0.89	27/46
No	40/70	1.1 (–15.0 to 17.3)	1.02 (0.77 to 1.36)		38/75	–5.3 (–21.3 to 10.6)	0.91 (0.67 to 1.22)		42/75
Age									
<36 yr	45/76	–3.1 (–18.6 to 12.3)	0.95 (0.74 to 1.23)	0.90	41/79	–10.4 (–25.9 to 5.0)	0.83 (0.63 to 1.10)	0.44	48/77
≥36 yr	22/47	–0.9 (–21.4 to 19.6)	0.98 (0.64 to 1.51)		20/41	1.1 (–20.2 to 22.3)	1.02 (0.66 to 1.59)		21/44
No. of miscarriages									
≥3	35/73	–3.4 (–19.6 to 12.8)	0.93 (0.67 to 1.29)	0.85	32/71	–6.3 (–22.5 to 10.0)	0.88 (0.63 to 1.23)	0.92	38/74
2	32/50	–2.0 (–20.9 to 17.0)	0.97 (0.72 to 1.30)		29/49	–6.8 (–26.1 to 12.5)	0.90 (0.66 to 1.22)		31/47

* Absolute differences in live-birth rate and relative risks were calculated for the comparison between patients receiving aspirin plus nadroparin (combination-therapy group) and the placebo group and between the aspirin-only group and the placebo group.
† A total of 302 of 364 women (83.0%) underwent a complete evaluation for inherited thrombophilia.

We used a broad definition of recurrent miscarriage (i.e., two or more miscarriages), in accordance with the definition used by the American College of Obstetrics and Gynecology.[28] Although the use of this definition may have diluted the results (as compared with the use of a definition of three or more miscarriages),[29,30] the characteristics of our study patients, as well as the live-birth rate observed among women in the placebo group, were consistent with those of other large cohorts in which no intervention was provided.[12,31] We also found no significant benefit of combination therapy or aspirin alone in subgroup analyses that were stratified according to the number of miscarriages.

Likewise, we found no significant benefits in other subgroups, including women with inherited thrombophilia (who might be most likely to benefit from treatment with heparin or aspirin[8,15,32]), although our study was not powered to assess subgroup effects. The possibility that one or both of these interventions might be beneficial in such women warrants further study in adequately powered, controlled trials. The prevalence of inherited thrombophilia among the women in our study population was 16%, which makes us confident that there was no selective referral of women without thrombophilia.[33]

Recently, in two randomized trials, investigators assessed the benefits of low-molecular-weight heparin in women with recurrent miscarriage.[34,35] In one trial involving 170 women with unexplained recurrent miscarriage, live-birth rates were significantly higher among women who were assigned to receive enoxaparin than among those assigned to receive placebo (81% vs. 48%).[34] In another study, involving 340 women who received enoxaparin or no treatment, the reported miscarriage rates were 5% and 11%, respectively.[35] However, methodologic limitations (i.e., a lack of blinding[35] or uncertain blinding procedures,[34] a high rate of loss to follow-up,[34] and a lack of prospective trial registration) and differences in the characteristics of the study populations (age[34,35] and number of previous miscarriages[35]) and the specific intervention make it difficult to compare the results of these trials with our findings.

In conclusion, our findings do not support the hypothesis that either combination therapy with aspirin and nadroparin or aspirin alone improves the chance of a live birth for women with unexplained recurrent miscarriage.

Supported by a grant (945-27-003) from the Netherlands Organization for Health Research and Development and a grant from GlaxoSmithKline. Study drugs (aspirin and placebo) were packaged and donated by Meda Pharma.

Dr. Büller reports receiving consulting and lecture fees from GlaxoSmithKline and Pfizer; and Dr. Middeldorp, consulting and lecture fees and grant support from GlaxoSmithKline. No other potential conflict of interest relevant to this article was reported. Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank all the women who participated in this study and the gynecologists who referred patients for inclusion; Dr. M.H. Prins of Maastricht University Medical Center and Dr. W.M. Ankum of the Academic Medical Center for their participation on the data and safety monitoring board; Dr. M. DiNisio of the University of Chieti for his contribution to the development of the study protocol; and the following physicians from the participating centers for their aid in recruitment of patients: E.M. Kaaijk and P.A. Flierman, Onze Lieve Vrouwe Gasthuis; L.L. Peeters, Maastricht University Medical Center; J. van der Meer (deceased) and J.J.H.M. Erwich, University Medical Center Groningen; G.J.E. Oosterhuis, Medisch Spectrum Twente Hospital Group, Enschede; S. Lunshof, Amphia Hospital, Breda; and I. Ruhe, Flevo Hospital, Almere; and W. Balliel, M. Knol, C. Verhoeven, M. Kuit-van Harten, L. van den Nieuwengiezen-Abdoellkariem, G.R. Sordam, R. Lagrand, and E. van den Boogaard for their help in the collection of data.

REFERENCES

1. Rai R, Regan L. Recurrent miscarriage. Lancet 2006;368:601-11.
2. ESHRE Capri Workshop Group. Genetic aspects of female reproduction. Hum Reprod Update 2008;14:293-307.
3. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:839-54.
4. Empson M, Lassere M, Craig J, Scott J. Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. Cochrane Database Syst Rev 2005;2:CD002859.
5. Farquharson RG, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment. Obstet Gynecol 2002;100:408-13. [Erratum, Obstet Gynecol 2002;100:1361.]
6. Rai R, Cohen H, Dave M, Regan L. Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). BMJ 1997;314:253-7.
7. Quenby S, Farquharson RG, Dawood F, Hughes AM, Topping J. Recurrent miscarriage and long-term thrombosis risk: a case-control study. Hum Reprod 2005;20:1729-32.
8. Rodger MA, Paidas M, McLintock C, et al. Inherited thrombophilia and pregnancy complications revisited. Obstet Gynecol 2008;112:320-4.
9. Tulppala M, Marttunen M, Söderstrom-Anttila V, et al. Low-dose aspirin in prevention of miscarriage in women with unexplained or autoimmune related recurrent miscarriage: effect on prostacyclin and thromboxane A2 production. Hum Reprod 1997;12:1567-72.
10. Dolitzky M, Inbal A, Segal Y, Weiss A, Brenner B, Carp H. A randomized study of thromboprophylaxis in women with unexplained consecutive recurrent miscarriages. Fertil Steril 2006;86:362-6.
11. Kaandorp S, Di Nisio M, Goddijn M, Middeldorp S. Aspirin or anticoagulants for treating recurrent miscarriage in

women without antiphospholipid syndrome. Cochrane Database Syst Rev 2009;1:CD004734.
12. Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. Hum Reprod 1999;14:2868-71.
13. Freundl G, Godehardt E, Kern PA, Frank-Herrmann P, Koubenec HJ, Gnoth C. Estimated maximum failure rates of cycle monitors using daily conception probabilities in the menstrual cycle. Hum Reprod 2003;18:2628-33.
14. Gnoth C, Godehardt D, Godehardt E, Frank-Herrmann P, Freundl G. Time to pregnancy: results of the German prospective study and impact on the management of infertility. Hum Reprod 2003;18:1959-66.
15. Brenner B. Antithrombotic prophylaxis for women with thrombophilia and pregnancy complications — Yes. J Thromb Haemost 2003;1:2070-2.
16. Rai R, Backos M, Elgaddal S, Shlebak A, Regan L. Factor V Leiden and recurrent miscarriage-prospective outcome of untreated pregnancies. Hum Reprod 2002;17:442-5.
17. Gilchrist DM, Livingston JE, Hurlburt JA, Wilson RD. Recurrent spontaneous pregnancy loss: investigation and reproductive follow-up. J Reprod Med 1991;36:184-8.
18. Regan L, Braude PR, Trembath PL. Influence of past reproductive performance on risk of spontaneous abortion. BMJ 1989;299:541-5.
19. Christiansen OB, Nybo Andersen AM, Bosch E, et al. Evidence-based investigations and treatments of recurrent pregnancy loss. Fertil Steril 2005;83:821-39.
20. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. BMJ 2000;320:1708-12.
21. Knudsen UB, Hansen V, Juul S, Secher NJ. Prognosis of a new pregnancy following previous spontaneous abortions. Eur J Obstet Gynecol Reprod Biol 1991;39:31-6.
22. Altman DG, Bland JM. Interaction revisited: the difference between two estimates. BMJ 2003;326:219.
23. Infante-Rivard C, David M, Gauthier R, Rivard GE. Lupus anticoagulants, anticardiolipin antibodies, and fetal loss: a case–control study. N Engl J Med 1991;325:1063-6.
24. Lockshin MD. Pregnancy loss in the antiphospholipid syndrome. Thromb Haemost 1999;82:641-8.
25. Laude I, Rongières-Bertrand C, Boyer-Neumann C, et al. Circulating procoagulant microparticles in women with unexplained pregnancy loss: a new insight. Thromb Haemost 2001;85:18-21.
26. Isermann B, Sood R, Pawlinski R, et al. The thrombomodulin-protein C system is essential for the maintenance of pregnancy. Nat Med 2003;9:331-7.
27. Bose P, Black S, Kadyrov M, et al. Heparin and aspirin attenuate placental apoptosis in vitro: implications for early pregnancy failure. Am J Obstet Gynecol 2005;192:23-30.
28. American College of Obstetricians and Gynecologists. ACOG practice bulletin: management of recurrent pregnancy loss: number 24, February 2001 (replaces technical bulletin number 212, September 1995). Int J Gynaecol Obstet 2002;78:179-90.
29. Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. Hum Reprod 2006;21:2216-22.
30. The investigation and treatment of couples with recurrent miscarriage. Guideline no. 17. London: Royal College of Obstetricians and Gynaecologists, 2003. (Accessed March 19, 2010, at http://www.rcog.org.uk/files/rcog-corp/uploaded-files/GT17RecurrentMiscarriage2003.pdf.)
31. Carp H, Feldman B, Oelsner G, Schiff E. Parental karyotype and subsequent live births in recurrent miscarriage. Fertil Steril 2004;81:1296-301.
32. Middeldorp S. Thrombophilia and pregnancy complications: cause or association? J Thromb Haemost 2007;5:Suppl 1:276-82.
33. Middeldorp S, Levi M. Thrombophilia: an update. Semin Thromb Hemost 2007;33:563-72.
34. Fawzy M, Shokeir T, El-Tatongy M, Warda O, El-Refaiey AA, Mosbah A. Treatment options and pregnancy outcome in women with idiopathic recurrent miscarriage: a randomized placebo-controlled study. Arch Gynecol Obstet 2008;278:33-8.
35. Badawy AM, Khiary M, Sherif LS, Hassan M, Ragab A, Abdelall I. Low-molecular weight heparin in patients with recurrent early miscarriages of unknown aetiology. J Obstet Gynaecol 2008;28:280-4.