Inherited thrombophilia and pregnancy complications

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Chapter 3

Long-term prognosis of women with recurrent miscarriage with or without inherited thrombophilia

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Abstract

Several studies evaluated the outcome of the first pregnancy after recurrent miscarriage. We aimed to determine the chance of a live birth over an extended follow-up period and to investigate the relevance of prognostic variables for time to live birth, including inherited thrombophilia. We collected data on pregnancies occurring in women who previously participated in the ALIFE study (ISRCTN 58496168), a randomized controlled trial investigating the efficacy of antithrombotic therapy on live birth in women with unexplained recurrent miscarriage. Follow-up data were available for 271 of 364 (74%) women, with a median follow-up of 84 months (15 to 169 months). The median time to a live birth was 19 months [9 to 105 months], and the cumulative probability of live birth, taking competing risks into account, was 15%, 55%, 77% and 81% after 1, 2, 5 and 10 years respectively. The cumulative probability of live birth was similar in women with and without inherited thrombophilia (hazard ratio 1.15, 95% confidence interval 0.77 – 1.72). The 81% cumulative incidence of live birth after 10 years is reassuring information for women with unexplained recurrent miscarriage with or without inherited thrombophilia. This is essential information when counselling couples with recurrent miscarriage.

Introduction

Recurrent miscarriage, as defined by the loss of two or more pregnancies before the fetus reaches a viable age, affects approximately 3% of women attempting to conceive.[1] Hematologists are often involved in the evaluation and management of these patients, especially when thrombophilia is present. Low-molecular-weight heparin and acetylsalicylic acid are prescribed to women diagnosed with recurrent miscarriage in antiphospholipid syndrome to increase the chance of live birth in a subsequent pregnancy.[2] Whether anticoagulants increase the chance of live birth in women with recurrent miscarriage and inherited thrombophilia is currently being evaluated (NTR3361, www.trialregister.nl).

When counselling couples with recurrent miscarriage, not only information regarding their chance of a live birth in a subsequent pregnancy is important, but also their chance of a live birth if more pregnancy attempts are made. Women with recurrent miscarriage are prone to undergo many diagnostic tests and ineffective therapeutic interventions.[3] If the prognosis

of women with recurrent miscarriage is good, this provides an opportunity to reduce burden and costs considerably.[4]

Several studies have evaluated the chance of live birth in the first pregnancy after diagnosis of recurrent miscarriage, with success rates depending on study population, onset of follow-up and definition of study outcome.[5] In a landmark study of 325 non-pregnant women with two or more unexplained miscarriages, 51% had an ongoing pregnancy beyond 24 weeks in the first subsequent pregnancy.[6] Recently, two large studies investigated the chance of live birth over time after recurrent miscarriage. In one study of 1250 women with two or more consecutive and unexplained pregnancy losses, 866 (69%) had at least one live birth.[7] However, the duration of follow-up was not described and remarkably, all women achieved at least one pregnancy. In the other study, 987 women with three or more consecutive losses before 22 weeks' gestation were followed during a maximum of 22 years, during which 67% had a live birth after 5 years, and 71.% after 15 years.[8] In this study, the cumulative incidence of live birth was calculated without considering age as a competing risk for live birth, which less accurately represents the probability of a live birth since women will not be able to have a live birth after reaching the age of 46 years. Furthermore, none of these studies investigated the effect of inherited thrombophilia.

The majority of women with RM prefers to obtaining novel information on RM as a part of supportive care.[9] The number of preceding miscarriages, timing of previous pregnancy losses (second or third trimester losses, including intra-uterine fatal deaths), previous live birth, maternal age, and an underlying factor such as hormonal or chromosomal abnormalities, and acquired thrombophilia, i.e. presence of antiphospholipid antibodies are considered determinants for prognosis.[10] Whether inherited thrombophilia increases the risk of miscarriage after recurrent miscarriage is controversial.[11-13]

We recently reported the time to conception and time to live birth in the first pregnancy of 251 women with unexplained recurrent miscarriage who had become pregnant in the ALIFE study.[14/15] Time to conception was about 2-fold shorter in women who carried Factor V Leiden as compared to non-carriers, whereas the median time to live birth was 102 weeks. Here, we report the long-term prognosis over an extended follow-up period, after diagnosis of unexplained recurrent miscarriage. Furthermore, we investigated the role of potential prognostic variables, including inherited thrombophilia.

Materials and methods

Study population and design

The study population consisted of women who had participated in the ALIFE study (ISRCTN 58496168), as reported previously.[15] In short, from 2004 through 2008, 364 women with two or more unexplained miscarriages before 20 weeks' gestation were randomized to receive either low-molecular-weight heparin (LMWH) plus acetylsalicylic acid (ASA), ASA only or placebo (for ASA). At time of randomization, they were either attempting to conceive or less than six weeks pregnant. Unexplained recurrent miscarriage was diagnosed in case of absence of abnormal parental karyotype, significant intrauterine abnormalities, lupus anticoagulant or anti-cardiolipin IgG and IgM antibodies, and abnormal fasting level of homocysteine. Between November 2012 and September 2014, we sent information on the study and a request for participation by postal mail, and subsequently contacted women by phone to collect follow-up data. Data on all pregnancies after the ALIFE study including dates and outcomes were collected using a predefined case record form. Only for women who were not reached after multiple attempts but for whom medical charts were available in the Academic Medical Center (the central study centre), medical charts were reviewed.

We defined the date of the last pregnancy *before* randomization in ALIFE as baseline (start of follow-up).

Outcome

The primary outcome was time to birth of a living neonate in the period from the last pregnancy prior to randomization in the ALIFE study until the end of follow-up.

Statistical analyses

The probability of a live birth was estimated using a competing risks analysis, where reaching the age of 46 years was considered as a competing risk for live birth. Data were censored when a live birth had not occurred or the age of 46 had not been reached before the end of follow-up.

We used data of women who participated in the follow-up study (primary analysis). We also performed a secondary analysis, including all women who participated in the ALIFE study (n=364). For women without available follow-up data, only data available from their participation in the ALIFE study were included. In the secondary analysis, data of women of whom no follow-up data were available were censored when a miscarriage, extra-uterine pregnancy or termination of pregnancy occurred in the ALIFE study period, when pregnancy was not reached within 24 months after randomization in the ALIFE study or at July 2009 (end of the ALIFE study), whichever came first. For women who dropped out, were lost to follow-up in the ALIFE study or who were not reached in follow-up, data were censored at the timing of drop out (if known) or at 1 year post-randomization. To investigate predictors of time to live birth, data of women of whom follow-up data were available were used. We first explored the relative prognostic significance of maternal age (age at the time of the last pregnancy before ALIFE, as categorical covariate (categorized as age younger than 26, 26 to 30, 31 to 35, 36 to 40, and 41 years or older), the number of preceding miscarriages (two versus three or more miscarriages as dichotomous covariate), previous live birth (yes or no) and inherited thrombophilia (presence of any form of inherited thrombophilia, yes or no; and Factor V Leiden separately, yes or no) for time to live birth in univariate analyses. Next, a multivariable proportional hazards model corrected for competing risks was performed, to evaluate the individual contributions of the predictors. Inclusion in the final model was determined by backward stepwise elimination, with a probability of F-to-remove ≥0.2.

Statistical analyses were performed using SPSS version 20.0 software and using the R statistical package version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). Two-sided probability values of <0.05 were considered statistically significant.

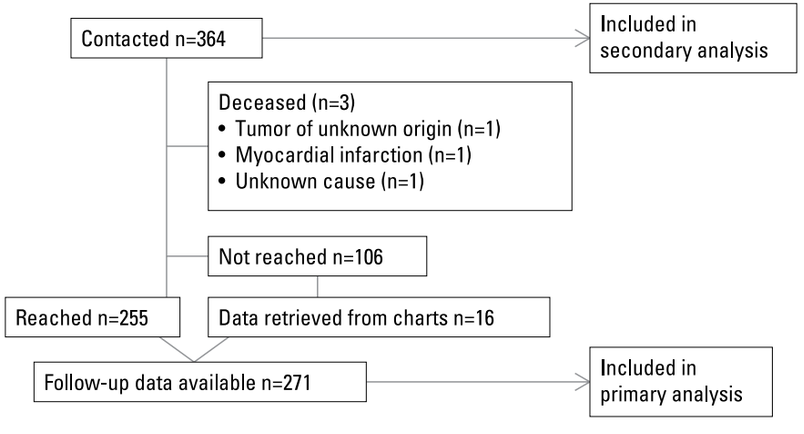
Ethics

The ALIFE study was approved by the local ethics committee (ethics registration no. 02/173) and all women provided written informed consent. For this follow-up study, the local ethics committee of the Academic Medical Center did not deem formal assessment necessary due to the nature of the questionnaires (waiver registration no. 2011_336#C20121219)

Results

A flow chart of the study population is depicted in **FIGURE 1**. Of 364 participants in the ALIFE study, 3 (0.8%) women had died after participation in the study, and follow-up data were available for 271 (74%) women, including 16 women of whom additional follow-up data were retrieved from medical charts. Of the 167 women who did not have a live birth during ALIFE, follow-up data were available for 113 (68%) women. Baseline characteristics for the full cohort and for women of whom follow-up data were available are summarized separately (**TABLE 1**). The mean age of the full cohort at baseline was 32.7 (range 20-42) years and the median time of follow-up since last pregnancy prior to randomization was 7 (range 1 to 14) years for women of whom follow-up data were obtained.

FIGURE 1 - *Flow chart of study population.*



The flow chart indicates which women were included in the primary and secondary analysis.

TABLE I - *Baseline characteristics of women with recurrent miscarriage.*

	n=271
Age (years), mean (SD)	32.7 (4.5)
Body mass index (kg/m²), mean (SD) *	25.0 (4.7)
Inherited thrombophilia, n (%) †	41 (17.9)
Factor V Leiden	19 (7.1)
Prothrombin mutation	3 (1.1)
Protein S deficiency	14 (6.0)
Protein C deficiency	5 (1.9)
Antithrombin deficiency	4 (1.6)
Pregnancies	
Number of previous miscarriage, median [range] ‡	3 [1-12]
Women with 2 previous miscarriages, n (%)	104 (38%)
Women with three or more previous miscarriages, n (%)	167 (62%)
Women with losses later than 12 weeks' gestation, n (%)	84 (31%)
Women who had a previous live birth, n (%)	105 (39%)

* Measured at time of randomization in ALIFE study. BMI-data were available for 258 women.
† Among patients who were evaluated for inherited thrombophilia, deficiencies were defined as <70% of normal activity for protein C, less than 65% for total protein S and less than 80% for antithrombin. Results of complete inherited thrombophilia testing were available for 225 women, ranging per thrombophilic factor from n=232 to n=268).
‡ 4 women were included early in the ALIFE study because of 1 miscarriage <20 weeks' gestation and 1 intra-uterine fetal death. Thereafter, the study protocol was amended, limiting the inclusion criteria to 2 miscarriages before 20 weeks' gestation.

Live birth and time to live birth

In the primary analysis of 271 women with complete follow-up data, 213 (79%) achieved a live birth (**TABLE II**). Of these, 158 (58%) had a live birth during their participation in the ALIFE study, whereas 55 women (20%) had at least one live birth thereafter. The number of pregnancies calculated from the start of follow-up ranged from 0 to 12, with 10 women who had 6 or more pregnancies. The median time to live birth was 19 months, ranging from 9 months to 105 months.
FIGURE II shows the cumulative probability of live birth, taking into account competing risks. The probabilities of live birth were 15%, 55%, 77% and 81% after 1, 2, 5 and 10 years respectively, with only limited increase of the probability after 4 years.
In the secondary analysis, also including data of women of whom only data during their participation in the ALIFE were available, the cumulative probability of live birth did not differ materially from the primary analyses (increasing from 15%, 54%, 77%, to 81% after 1, 2, 5 and 10 years respectively.

Determinants of prognosis

Results of the univariate and multivariable analyses of the association between patient characteristics and time to live birth are shown in **TABLE III**. Although age was not significantly associated with time to live birth in the univariate analysis, a trend for an inverse association was observed between age and time to live birth when the age categories (31-35, 36-40, ≥41 years) were compared to the reference category (26-30 years). A history of three or more previous miscarriages was significantly associated with a longer time to live birth in univariate analysis when compared to a history of two miscarriages.
In the multivariable analysis with all potential prognostic variables in the model (full model), only the number of previous miscarriages (HR 0.75, 95% CI 0.57 – 0.97) was an independent predictor of time to live birth, while a previous live birth and presence of any form of inherited thrombophilia were not significantly associated. After stepwise backward elimination only the number of previous miscarriages remained in the model, corresponding to live birth probabilities of 15%, 63%, 86% and 88% after 1, 2, 5 and 10 years in women with two miscarriages versus 15%, 50%, 71% and

73% in women with three or more miscarriages (**FIGURE II-B**).
Factor V Leiden, when tested independently of the other forms of inherited thrombophilia, was not associated with time to live birth in both the univariate analysis (HR 0.90, 95% CI 0.52 – 1.57) and multivariable analysis.

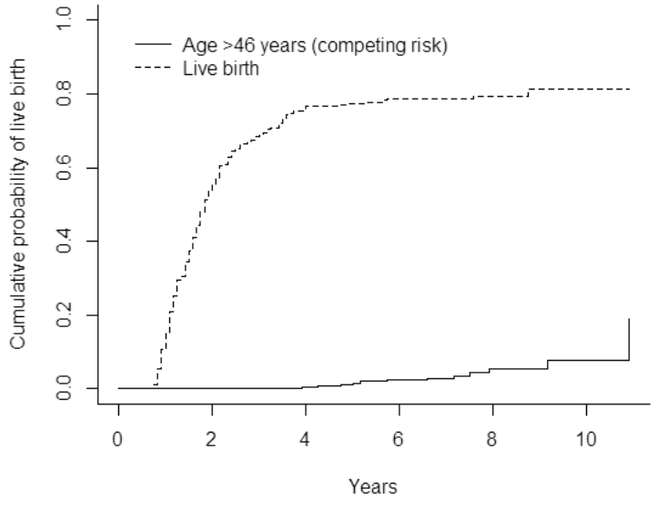
TABLE II - *Number of pregnancies and live births during follow-up in women with recurrent miscarriage.*

	Complete follow-up available n=271 n (%)	Full cohort ALIFE study n=364 n (%)
Number of subsequent pregnancies		
0	20 (7.4)	46 (12.6)
1	77 (28.4)	144 (39.6)
2	88 (32.5)	88 (24.2)
3	38 (14.0)	38 (10.4)
4	26 (9.6)	26 (7.1)
5	12 (4.4)	12 (3.3)
6	4 (1.5)	4 (1.1)
7	4 (1.5)	4 (1.1)
8	1 (0.4)	1 (0.3)
12	1 (0.4)	1 (0.3)
Frequency of live births		
0	58 (21)	112 (31)
1	114 (42)	153 (42)
2	88 (32)	87 (24)
3	10 (4)	10 (3)
4	2 (0.7)	2 (0.5)
Number of live births	213	252
Pregnancy in which first live birth was achieved n (%) (cumulative %)		
1st	167 (62) (62)	206 (57) 57)
2nd	30 (11) (73)	30 (8) (65)
3rd	8 (3) (76)	8 (2) (67)
4th	5 (2) (78)	5 (1) (68)
5th	2 (0.7) (79)	2 (0.5) (69)
6th	1 (0.4) (79)	1 (0.3) (69)

Percentage totals do not always meet 100% due to rounding.

FIGURE II - *Cumulative probability of a first live birth.*

A



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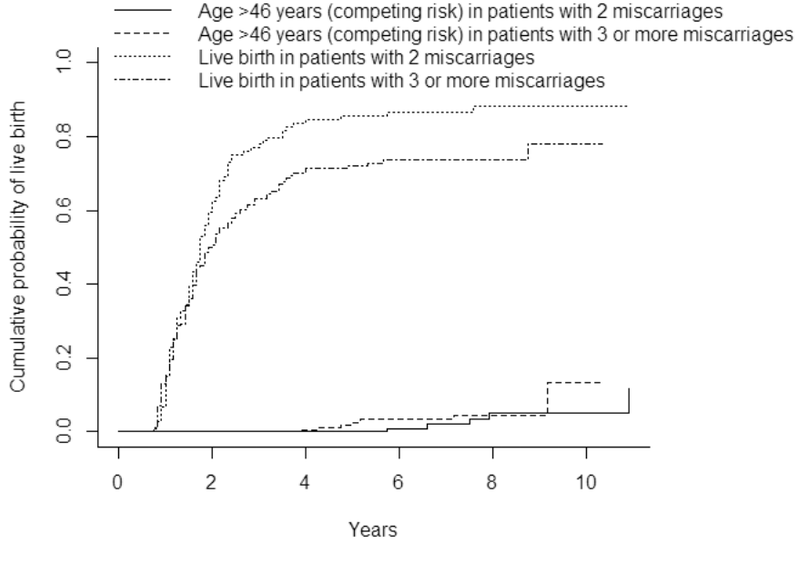

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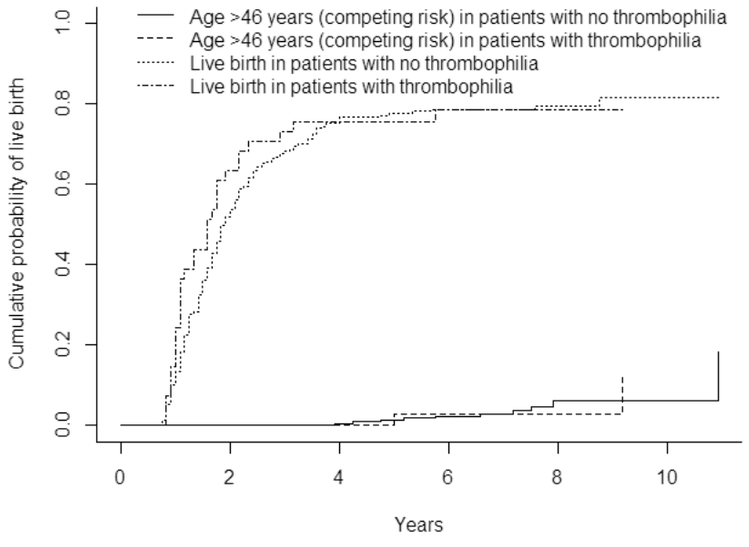

(A) Cumulative probability of a first live birth or competing risk for live birth (i.e. reaching the age of 46) in 271 women with unexplained recurrent miscarriage. (B) Cumulative probability of a first live birth or competing risk for live birth (i.e. reaching the age of 46) after unexplained recurrent miscarriage; compared between women with 2 vs 3 or more previous miscarriages. (C) Cumulative probability of a first live birth or competing risk for live birth (i.e. reaching the age of 46) after unexplained recurrent miscarriage; compared between women with inherited thrombophilia vs no inherited thrombophilia. t=0 indicates the start of follow-up, defined as the date of the last pregnancy before randomization in the ALIFE study.

TABLE III - *Proportional hazards for live birth for prognostic variables corrected for competing risks in women with unexplained recurrent miscarriage (n=271).*

	Univariate	Model 1*	Model 2†
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Age (years)			
Younger than 26	0.78 (0.47 – 1.30)	0.74 (0.45 – 1.21)	
26 to 30	Reference	Reference	--
31 to 35	0.79 (0.57 – 1.10)	0.80 (0.58 – 1.11)	
36 to 40	0.67 (0.46 – 0.97)	0.66 (0.46 – 0.97)	
Older than 40	0.24 (0.06 – 0.86)	0.27 (0.07 – 0.99)	
No. of previous miscarriages			
2	Reference	Reference	Reference
3 or more	0.73 (0.56 – 0.95)	0.75 (0.57 – 0.97)	0.73 (0.56 – 0.95)
Previous live birth			
No	Reference	Reference	--
Yes	0.93 (0.70 – 1.24)	1.01 (0.75 – 1.34)	
Inherited thrombophilia‡			
No	Reference	Reference	--
yes	1.15 (0.77 – 1.74)	1.15 (0.77 – 1.72)	

HR hazard ratio; CI confidence interval; * Full model. † After stepwise backward elimination. ‡ Results of complete inherited thrombophilia testing were available for 302 women (47 women tested positive for at least one of the thrombophilic factors). For this analyses all women with no or incomplete inherited thrombophilia test result were considered negative for inherited thrombophilia.

Discussion

Our study indicates that women with two or more unexplained miscarriages have a cumulative probability of live birth of 81%. An increasing number of previous miscarriages (i.e. three or more) was associated with a longer time to live birth when compared to a history of two miscarriages. Furthermore, we observed that time to live birth in women with inherited thrombophilia does not differ from women without inherited thrombophilia.

To our knowledge, this is the first study evaluating live birth and time to live birth during extended follow-up of up to 14 years in a cohort of women with unexplained recurrent miscarriage, who were not already pregnant at the start of follow-up. The high cumulative probability in our study as compared to other studies may be explained by differences in study populations, or differences in statistical methods. A previous study in 987 women found a lower live birth rate of 71% after 15 years, but included women with both explained and unexplained recurrent miscarriage and did not include women with two miscarriages.[8] In the present analysis, we corrected for the competing risk of reaching the age at which achieving a live birth was considered impossible, i.e. 46 years. Not taking this into consideration in the analysis might lead to an underestimation of the result.
Our observation that an increasing number of miscarriages adversely affects the probability of live birth, is in line with the before mentioned study which reported a hazard ratio of live birth 5 years after consultation of 0.55 (95% CI 0.41 – 0.74) for women with six or more miscarriages when compared to women with three previous miscarriages.[8]
Remarkably, our study indicates that maternal age is not predictive of time to live birth. Although in the univariate analyses older women had a worse prognosis, this was not substantiated in the multivariable model.
In the same cohort, taking first subsequent pregnancies only into account, we found that Factor V Leiden was associated with shorter time to conception.[14] In the current analyses, neither Factor V Leiden independently, nor the presence of any thrombophilic factor were associated with time to live birth. It could be hypothesized that the detrimental effect of Factor V Leiden in terms of an increased risk of miscarriages does not translate to a longer time to live birth, potentially because of a shorter time to conception.[16/17]

Three women (0.8%) had died during the follow-up period. One woman died at 42 years of age of a carcinoma of unknown primary origin, the second woman died shortly after she had an anterior myocardial infarction at 35 years of age, and the third was found in her home (30 years of age), with unknown cause of death. Although this is an unusual high number of deaths in such a young population, we have no study-related explanation for this.

This study has some limitations. Follow-up data were not available for all 364 women who participated in the ALIFE study. However, because of the high response rate of 74% and as both the baseline characteristics as well as the cumulative probability of live birth were the same for the group of women reached in follow-up (n=271) and for the total cohort (n=364) (i.e. 81%), we believe this is a representative sample. Furthermore, whether follow-up was obtained or not appeared to be random as it was mostly due to loss of contact information. Very few women refused participation in the questionnaire study. Unfortunately, reliable data on treatment during pregnancies could not be obtained, as women often did not recall if they were treated and if so, during which pregnancy they had received treatment. This information would have been of value, and should be collected in a prospective study, especially as for women with inherited thrombophilia, it is unclear if treatment with anticoagulants increases their chance of live birth.[18]
We believe that the current approach has provided valuable information. Instead of using medical charts, which can be incomplete in case women received care of their pregnancies in different hospitals, for the vast majority we obtained data from the women themselves.
The population investigated in this study consists of women who participated in the ALIFE study. This may be considered a limitation to the generalisability of the results. However, as the ALIFE-study population appeared representative of the recurrent miscarriage-clinic patients, participation in the study was offered to each candidate and refusal of participation did not appear associated with a better or worse prognosis, we are confident that the results apply to all women with unexplained recurrent miscarriage. A full thrombophilia screen was available for 229 women (85%) and only 41 women tested positive for any inherited thrombophilia (18%), of whom 19 for Factor V Leiden. As women with missing data were considered negative for the thrombophilic factor concerned in the analyses the actual prevalence was likely higher and this may have led to an underestimation of a potential effect of inherited thrombophilia.

The cumulative probability of live birth during a follow-up period of 10 years was 81% for all women and 73% for women with three or more miscarriages. This can be interpreted as the chance of live birth in case a woman is granted another 10 fertile years during which she keeps attempting to conceive. In conclusion, this study indicates that the prognosis of women with unexplained recurrent miscarriage is generally good and not affected by the presence of inherited thrombophilia.

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