Identify, appraise and individualize: clinical practice and prediction models in recurrent pregnancy loss

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PART II

PROGNOSIS IN RECURRENT PREGNANCY LOSS

6

CHAPTER 6

DEFINING RECURRENT PREGNANCY LOSS; ASSOCIATED FACTORS AND PROGNOSIS IN COUPLES WITH TWO VS THREE OR MORE PREGNANCY LOSSES

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ABSTRACT

RESEARCH QUESTION

The definition of recurrent pregnancy loss (RPL) differs internationally. The European Society of Human Reproduction and Embryology (ESHRE) defines RPL as two or more pregnancy losses. Different definitions lead, however, to different approaches to care for couples with RPL. This study aimed to determine whether the distribution of RPL-associated factors was different in couples with two versus three or more pregnancy losses. If a similar distribution were found, couples with two pregnancy losses should be eligible for the same care pathway as couples with three pregnancy losses.

DESIGN

This single-centre, retrospective cohort study investigated 383 couples included from 2012 to 2016 at the Leiden University Medical Centre RPL clinic. Details on age, body mass index, smoking status, number of pregnancy losses, mean time to pregnancy loss and performed investigations were collected. The prevalence of uterine anomalies, antiphospholipid syndrome, hereditary thrombophilia, hyperhomocysteinemia, chromosomal abnormalities and positive thyroid peroxidase antibodies were compared in couples with two versus three or more pregnancy losses.

RESULTS

No associated factor was found in 71.5% of couples with RPL. This did not differ statistically between couples with two versus three or more pregnancy losses (73.6% versus 70.6%; p=0.569). The distribution of investigated causes did not differ between the two groups.

CONCLUSIONS

As the distribution of associated factors in couples with two versus three or more pregnancy losses is equal, couples with two pregnancy losses should be eligible for the same care pathway as couples with three. This study supports ESHRE's suggestion of including two pregnancy losses in the definition of RPL.

INTRODUCTION

Miscarriage is defined as the spontaneous loss of conception before the 24th week of gestation, and occurs in approximately 15% of pregnancies (1). Recurrent pregnancy loss (RPL) was recently defined by the "European Society of Human Reproduction and Embryology" (ESHRE) as two or more pregnancy losses (1). This new definition includes biochemical pregnancies and pregnancies of unknown location. The prevalence of a spontaneous loss could be higher using the ESHRE definition (2). However, different definitions are used in different guidelines and different countries (1, 3).

Factors generally accepted to be associated with RPL include uterine malformations, maternal antiphospholipid syndrome (APS), maternal thrombophilia, endocrine disease (such as diabetes and presence of thyroid antibodies), autoimmune diseases and parental structural chromosomal abnormality (4). Factors contributing to RPL are increased female age, female weight (obesity and underweight) and lifestyle factors such as smoking, caffeine and alcohol intake (5). Unfortunately, in approximately 50% of couples no associated factor can be identified (6).

In the discussion of defining RPL, Boogaard et al stated that the number of preceding miscarriages is not associated with the risk of APS and that APS testing should also be considered for women with two or more pregnancy losses (7). The same authors showed that the probability of carrier status of a structural chromosomal abnormality is not only influenced by the number of preceding miscarriages. Low maternal age at second miscarriage, a history of two or more miscarriages in a brother or sister of either partner, and a history of two or more miscarriages in the parents of either partner do increase the probability of carrier status (8).

However, whether the risk related to the above-mentioned associated factors is different for couples with two versus three or more pregnancy losses remains to be elucidated. This is important to know because if a similar distribution of these factors were found, couples with two pregnancy losses should be eligible for the same care pathway as couples with three or more pregnancy losses. It could also be of use in clinical research as varying definitions are currently used. In addition, it is not known whether the risk of a subsequent miscarriage after two (non-

)consecutive losses is similar to the risk after three miscarriages (9, 10). From a patient's perspective, this question might be even more important.

The objective of this study was therefore to determine whether the distribution of RPL associated factors is different in couples with two vs three or more pregnancy losses. In addition, mean gestational age at time of miscarriage and chance of future ongoing pregnancy were assessed.

MATERIALS AND METHODS

This single-centre retrospective cohort study included couples with a history of RPL who were evaluated at the RPL clinic of the Leiden University Medical Centre (LUMC) between November 2012 and October 2016. The clinic investigates associated factors in couples with RPL and provides support during subsequent pregnancies, with weekly ultrasounds from the sixth week of gestational age until the 12th week.

RPL was defined as at least two or more pregnancy losses before 24 weeks of gestational age, including non-visualized pregnancies and non-consecutive pregnancy losses. Ectopic and molar pregnancies were not included in the study definition. An ongoing pregnancy was defined as a pregnancy continuing after the 10th gestational week.

A database was created in SPSS (IBM Inc., New York, USA version 23) to include data from the electronic patient records on intake of each new patient who visited the clinic in the defined period. Clinical data prior to 2013 originated from paper files. At the first intake appointment, a thorough medical and obstetric questionnaire was completed and women were evaluated for uterine anomalies, APS (anticardiolipin antibodies, lupus anticoagulant and anti-β2-glycoprotein-I), hereditary thrombophilia (protein C, protein S or antithrombin III deficiency, activated protein C [APC] resistance [due to the factor V Leiden mutation] or factor II mutation), hyperhomocysteinemia and parental karyotyping. Parental karyotyping was evaluated according to a priori probability (8), APS and maternal thrombophilia testing was performed at least 12 weeks apart from a patient's last miscarriage. Details of the procedure for evaluating the causes of RPL are described below. Anti-thyroid peroxidase (anti-TPO) concentrations were measured in patients interested in participating in a

clinical trial (T4-LIFE) studying the effectiveness of levothyroxine supplementation in women with RPL and thyroid autoimmunity (11).

The first day of the last menstrual period was used to calculate the gestational age of the miscarriage as exact methods, such as ultrasonography, were not available for all women. The outcome and parameters of the subsequent pregnancy were documented, and information collected from medical records outside the hospital was added to the patient file and database. The results of investigation of RPL for all couples were discussed in an RPL team. Consensus was achieved in diagnosing possible causes of the RPL. RPL was declared unexplained when none of the investigated causes was found in couples.

EVALUATION OF CAUSES OF RPL

Congenital uterine anomalies were detected by 3D ultrasound in the luteal phase. The 3D ultrasound was performed by a sonographer specialised in 3D ultrasounds. Diagnostic methods, for example hysteroscopy, used outside the centre were added to the database. The ESHRE/ESGE classification of female tract congenital anomalies was used to classify uterine anomalies (18). Uterine anomalies were already being diagnosed in coherence with the guideline before its publication.

APS was diagnosed if at least one clinical and one laboratory criterion was present (19). The clinical criteria were either vascular thrombosis or pregnancy morbidity (including unexplained RPL); laboratory criteria were the presence of lupus anticoagulant, elevated titres of anticardiolipin IgG and/or IgM antibody (≥40 U/ml), or anti-β2 glycoprotein-I IgG and/or IgM antibody (≥17 U/ml) at two different time points at least 12 weeks apart. Women with a positive diagnosis were given low molecular weight heparin (2850 IU daily), starting from a positive pregnancy test up to 24 h before labour. Aspirin (80 mg daily) was added once a foetal heartbeat was detected and was continued up to 36 weeks' gestational age.

Abnormal results were recorded when antigen concentrations and activity levels of protein C were less than 66% and 64%, respectively. A free protein S antigen concentration less than 0.53 IU/ml and an antithrombin III antigen concentration less than 84% were considered abnormal. Factor II mutations (heterozygous/homozygous) were investigated, as was the factor

V Leiden mutation when the APC resistance ratio was lower than 0.91. Hyperhomocysteinemia was diagnosed when random homocysteine concentrations surpassed 15 mmol/l. If homocysteine concentrations were elevated, vitamin B6 (reference range 54–136 nmol/l) and B12 concentrations (reference range 150–700 pmol/l) were measured; supplements were given if the levels were too low. After 6 weeks, the homocysteine concentration was re-evaluated. If it was normal, couples could stop using contraceptives.

According to Franssen's risk table on chromosomal abnormalities, couples could be genetically tested for the presence of structural chromosomal abnormalities (8). A clinical geneticist evaluated both parents' karyotype and concluded whether significant abnormalities were present.

ETHICAL APPROVAL

The study protocol (reference number P11.196) was approved by the LUMC ethics committee (October 2015).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS Statistics version 23 (IBM, USA). To analyse differences between the groups, the independent samples t-test was used for continuous data. For categorical variables, Pearson's chi-squared test was used. If there was an expected count of less than 5 in 20% or more of all cells in SPSS, Fisher's exact test was used.

A linear by linear association chi-squared test was used to compare the chances of ongoing pregnancy in couples with different gestational ages. Binary logistic regression analysis was conducted to assess the influence of age and body mass index (BMI) on the chance of having a future ongoing pregnancy. BMI was used as a categorical variable, with BMI categories of less than 20 kg/m^2, over 25 to 30 kg/m^2 and over 30 kg/m^2 compared with the healthy range of 20–25 kg/m^2. Groups were assumed to differ significantly when the probability level was less than 0.05.

RESULTS

Over the study period in 2012 until 2016, 383 couples with at least two pregnancy losses visited the clinic and were assessed. The women's mean age was 33.7 years and mean BMI 25.1 kg/m^2. The mean gestational age of

the pregnancy loss at presentation was 7 weeks and 4 days. The percentage of women who smoked was 17.3%. Table 1 displays the baseline characteristics of the RPL population. Due to missing data, the number of couples used in calculating the baseline characteristics is given for BMI, mean gestational age, smoking rate and ongoing pregnancy rate.

Table 1 | Baseline characteristics

	Total	Two pregnancy losses	Three or more pregnancy losses	p-value+
Age (years), mean ± SD (n)	33.7 ± 4.7 (383)	33.6 ± 4.7 (107)	33.7 ±4.7 (276)	0.800
BMI (kg/m^2), mean ± SD (n)	25.1 ± 5.2 (332)	25.4 ± 5.2 (91)	25.0 ± 5.1 (241)	0.583
Prior live birth, % (n)	42.6 (163/383)	51.4 (55/107)	39.1 (108/276)	0.029
Gestational age* (weeks), mean ± SD (n)	7.6 ± 1.6 (324)	8.0 ± 1.7 (84)	7.4 ± 1.5 (240)	0.004
Smokers, % (n)	17.3 ± 3.8 (307/371)	16.8 ± 3.8 (17/101)	17.4 ± 3.8 (47/270)	0.896
Ongoing pregnancy rate, % (n)	83.5 (212/254)	91.5 (65/71)	80.3 (147/183)	0.031

SD: standard deviation
* Mean gestational age at time of miscarriage
+ Independent samples t-test

At the patients' initial visit, for the mean age of 33.7 years (n=383), the age range was 22–45 years. An independent t-test showed no statistically significant difference in age among women with two versus three or more pregnancy losses (mean age 33.6 ± 4.7 years (n=107) for two losses and 33.7 ± 4.7 years (n=276) for three or more losses; p=0.800). Mean BMI (kg/m^2) did not differ between women with two versus three or more losses (25.4 ± 5.2 versus 25.0 ± 5.1 kg/m^2; independent t-test, p=0.583), and neither did the smoking percentage (16.8 ± 3.8 versus 17.4 ± 3.8; chi-squared test, p=0.896).

The majority of women (98.2%, n=324) had a miscarriage before the 11th week of gestation. The mean gestational age at pregnancy loss was significantly higher in women with two versus three or more pregnancy losses (8.0 ± 1.7 (n=84) versus 7.4 ± 1.5 (n=240); independent t-test, p=0.004).

ASSOCIATED FACTORS OF TWO VS THREE OR MORE PREGNANCY LOSSES

At the initial visit, 27.9% (n=107) of the couples had two pregnancy losses and 72.1% (n = 276) had three or more pregnancy losses. Almost half of all couples (42.6%, n=163) had at least one live birth prior to or in between the pregnancy losses.

Table 2 summarizes the distribution of associated factors among these couples.

Table 2 | Distribution of associated factors

	Two pregnancy losses	Three or more pregnancy losses	Total	p-value[+]
Hyperhomocysteinemia, % (n)	2.0 (2/101)	1.1 (3/265)	1.4 (5/366)	0.619[*]
Antiphospholipid syndrome, % (n)	6.5 (7/107)	7.6 (21/276)	7.3 (28/383)	0.719[+]
Hereditary thrombophilia, % (n)	12.7 (13/102)	11.8 (31/263)	12.1 (44/365)	0.801[+]
Chromosomal abnormality, % (n)	2.9 (2/68)	2.9 (5/172)	2.9 (7/240)	1.000[*]
Anti-thyroid peroxidase, % (n)	9.0 (7/78)	10.7 (18/168)	10.2 (25/246)	0.674[+]
Uterine anomaly, % (n)	3.4 (3/88)	7.1 (17/239)	6.1 (20/327)	0.215[+]
Unexplained, % (n)	73.6 (78/106)	70.6 (190/269)	71.5 (268/375)	0.569[+]

* Fisher's exact test
\+ Chi-squared test

In 71.5% of 375 couples who were evaluated, RPL was unexplained. Due to missing data, we were not able to register a diagnosis for eight couples in the database. The prevalence of investigated factors in couples with two vs. three or more pregnancy losses was: uterine anomalies, 3.4% vs. 7.1% (p=0.215); APS, 6.5% vs. 7.6% (p=0.719); hereditary thrombophilia, 12.7% vs. 11.8% (p=0.801); hyperhomocysteinemia, 2.0% vs. 1.1% (p=0.619); chromosomal abnormalities, 2.9% vs. 2.9% (p=1.000); and positive TPO antibodies, 9.0% vs. 10.7% (p=0.674). The distribution of associated factors did not differ between the two groups. There was no statistically significant difference in the number of unexplained RPL (73.6% versus 70.6%; p=0.569) in couples with two versus three or more pregnancy losses.

PROGNOSTIC FACTORS

Of the 383 couples included in this study, 66.3% (n=254) continued to visit the LUMC with a new pregnancy after their RPL workup and treatment advice plan. The mean follow-up time was 37.7 ± 7.1 months. Of these couples, 83% (n=212) had an ongoing pregnancy during follow-up. Couples with two pregnancy losses had significantly more ongoing pregnancies compared with couples with three or more losses (91.5% vs. 80.3%; p=0.031; Table 1). This decrease in number of ongoing pregnancies continued when two, three or four or more losses were compared, as shown in Figure 1 (p=0.035 for overall decrease from two until four or more losses).

As the mean gestational age of miscarriage increased, an associated factor was more often identified (p=0.004) and the chance of having an ongoing pregnancy increased (p=0.005) (Figure 2). In contrast to couples with two losses, the association between the mean gestational age of the miscarriage and the chance of having an ongoing pregnancy was significantly different in couples with three or more pregnancy losses (chi-squared test, p=0.458 vs. p=0.035).

A greater number of women with a BMI of 25 kg/m^2 or less compared with a BMI of over 25 kg/m^2 had an unexplained cause of RPL (n=330, 72.3%, 138/191, vs. 60.4%, 84/139; p=0.024). This difference remained statistically significant in women with two pregnancy losses (n=90, 76.6%, 36/47, versus 51.2%, 22/43; p=0.012) but not in women with three losses (n=235, 70.4%, 100/142, vs. 63.4%, 59/93; p=0.263). The distribution of associated factors was not statistically significantly different in the two comparisons.

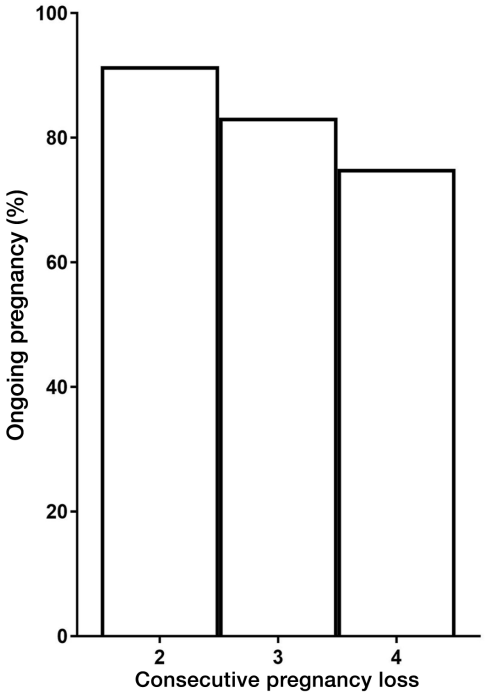

Figure 1 The chance of a future ongoing pregnancy in women with two, three or four or more pregnancy losses. Total, n = 254; two losses, n = 71; three losses, n = 119; four or more losses, n = 64. Chi-squared test, P = 0.035 for overall decrease from two until four or more losses.

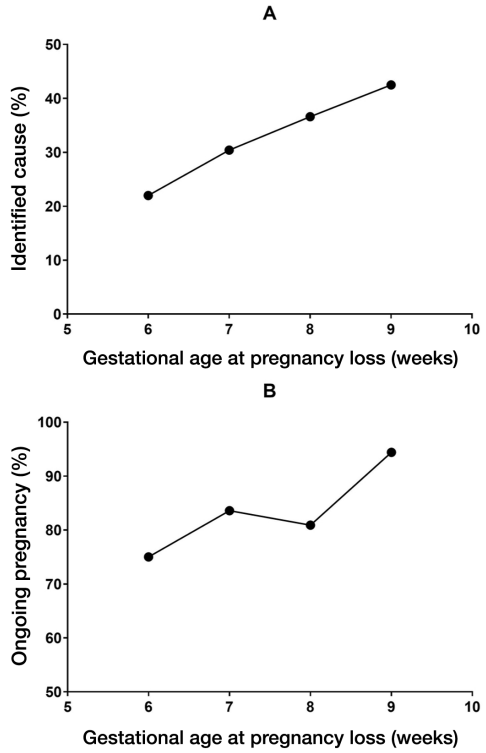

Figure 2 Prognostic factors. (A) Graph showing how often an associated factor was found in women with different mean gestational ages at the time of miscarriage. Chi-squared test, p=0.004 (for overall graph). (B) Graph showing the chance of a future ongoing pregnancy in women with different mean gestational ages at the time of miscarriage. Chi-squared test, p=0.005 (for overall graph).

Table 3 | Analysis of the influence of age (years) and BMI (kg/m^2) on the chance of having a future ongoing pregnancy in women followed up after their first visit

				95% CI	
	B ± SE	**p-value**	**OR**	**Lower**	**Upper**
Age	−0.023 ± 0.041	0.574	0.977	0.901	1.060
BMI 20–25		0.265			
BMI <20	0.655 ± 0.589	0.266	1.926	0.607	6.107
BMI >25 to 30	0.787 ± 0.470	0.094	2.197	0.875	5.521
BMI >30	0.698 ± 0.587	0.235	2.009	0.636	6.353
Constant	2.020 ± 1.415	0.153	7.539		

Body mass index (BMI) was used as categorical variable, in which the categories BMI <20 kg/m^2, over 25 to 30 kg/m^2 and over 30 kg/m^2 were compared with the healthy range of 20–25 kg/m^2.
B: unstandardized regression weight; OR, odds ratio for the correspondent variable with regard to the chance of having a future ongoing pregnancy.

The binary logistic regression analysis did not indicate an association between age, BMI and the chance of an ongoing pregnancy (Table 3). This did not change when the analysis was performed separately in women with two or with three or more pregnancy losses.

DISCUSSION

In this study, associated and prognostic factors were compared between couples with two and those with three or more pregnancy losses in 383 couples from the RPL cohort over a period during 2012–2016. Factors associated with RPL occurred with equal frequency in women with two versus three or more pregnancy losses.

A knowledge of similarities between couples with two versus three or more pregnancy losses is clinically relevant. A uniform definition is of importance to be able to standardize protocols as well as for a unified method in scientific research related to women with RPL. These women carry a burden of not being able to successfully reproduce, and early intervention with "tender, loving care" could reassure these couples. Reassurance could also be derived from information on the chances of future ongoing pregnancy.

In most cases (71.5%), no underlying associated factor of RPL was found. The most common factor was thrombophilia, occurring in 12.1% of RPL couples, followed by anti-TPO antibodies (10.2%), APS (7.3%), uterine malformations (6.1%), chromosomal abnormalities (2.9%) and hyperhomocysteinemia (1.4%). The prevalence of these associated factors was compared with other studies, and no striking differences were found (7, 12, 13). The most important difference is the inclusion of anti-TPO in the RPL workup in the current study. Anti-TPO is associated with RPL and the odds of pregnancy losses are increased in women with antibodies, even if they have normal thyroid function. Couples willing to participate in the T4-LIFE study, which investigates levothyroxine supplementation in women with RPL and thyroid autoimmunity in relation to live birth rate and pregnancy outcome, were assessed for the presence of autoantibodies directed against the thyroid gland.

In the current study, women with two versus three or more pregnancy losses were comparable in their characteristics in terms of age, BMI and smoking habit. The distribution of associated factors was equal in couples

with two versus three or more pregnancy losses, and a lack of cause was found as often in the two groups. The mean gestational age of the miscarriage was, however, significantly lower in the group with at least three pregnancy losses. It is well known that foetal genetic abnormalities occur frequently and can be the cause of a pregnancy loss (14, 15). In women with three or more pregnancy losses, these pregnancy losses could potentially be explained by foetal genetical abnormalities, leading to miscarriage at earlier gestational age. Although the result was highly statistically significant, the difference was one of only a few days, and one could argue the clinical relevance of this observation.

The chances of having a future ongoing pregnancy are relatively high even though this chance decreases with an increasing number of pregnancy losses. During follow-up, 83.5% of women were reported to have an ongoing pregnancy, which is in line with another cohort of women with unexplained RPL (16). Also in accordance with this, a subsequent pregnancy loss was seen to negatively influence the chance of a future ongoing pregnancy. In the current study, the chance of a future ongoing pregnancy was calculated in relation to the whole RPL population, whereas Brigham and colleagues calculated this chance in a population of women with unexplained RPL. The chance of a future ongoing pregnancy is, however, still relatively high (Figure 1), which could be a comforting thought for women suffering from RPL.

This study collected data from women who visited the RPL clinic between 2012 and 2016, even though the clinic opened in 2007. Data originating before 2013 were derived from paper files, so were less accessible than if electronic patient files had been used. Moreover, the data from the paper files were frequently incomplete, but adding this information through questionnaires could have led to recall bias. However, the data derived from paper files formed the minority of the collected data. Many women had been referred to the clinic having undergone several investigations elsewhere, meaning that information from those investigations was added to the database.

This is, for example, the case in the investigation of uterine anomalies. In other hospitals, general 2D ultrasound, hysteroscopy, hysterosalpingography, laparoscopy, magnetic resonance imaging or saline

infusion sonohysterography were used to diagnose anomalies, whereas the LUMC protocol uses a 3D ultrasound technique. This could have led to an underestimation of the prevalence of uterine anomalies in the current cohort.

New insights into hyperhomocysteinemia and RPL are presented in the latest ESHRE guideline. Before this guideline, hyperhomocysteinemia was part of the regular RPL investigations. The results of statistical testing did not, however, change when hyperhomocysteinemia was excluded from the analysis.

Another important limitation of this study is the small number of couples included. A larger cohort is needed to estimate the outcomes more precisely, especially for prognostic factors.

Jaslow and colleagues showed that the distribution of associated factors in women with RPL did not differ between two, three or four or more losses (12). In addition, Bashiri and co-workers showed that there were no differences between women with two versus three or more losses (13). Van Dijk and colleagues recently published a systematic review and meta-analysis evaluating the occurrence of abnormal test results in patients with two versus three or more pregnancy losses (17). They concluded that there was no difference in the prevalence in uterine abnormalities and APS, but that they could not exclude a difference in chromosomal abnormalities, inherited thrombophilia and thyroid disorders.

The current findings are in accordance with this review. Moreover, the current study adds to the body of evidence that there is no difference in inherited thrombophilia in women with two versus three or more pregnancy losses. In this cohort, the number of chromosomal abnormalities was equal, but numbers were small. With regard to thyroid diseases, anti-TPO results are equal in both groups in this study. Again, the number of patients with TPO antibodies is small, as the presence of these antibodies was, as mentioned above, only assessed in couples who were willing to participate in the T4-LIFE study.

CONCLUSION

This study assessed differences in factors associated with RPL in women with two versus three or more pregnancy losses in terms of the discussion of the definition of RPL. An equal distribution of associated factors was found in couples with two versus three or more pregnancy losses. The most recent ESHRE guideline advises defining RPL as starting from two pregnancy losses, and this study supports that definition.

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