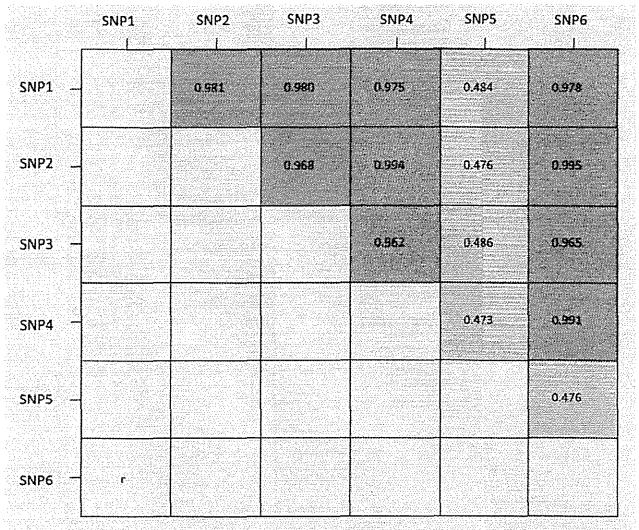
FIGURE 1



Linkage disequilibrium of the six *ANXA5* single-nucleotide polymorphisms (SNPs).

Hayashi. ANXA5 *SNPs in recurrent pregnancy loss. Fertil Steril 2013.*

frequency between patients and controls among the three haplotypes.

Of the 264 patients with RPL, 185 (70.1%) subsequently gave live births. Of the 79 aborted products, normal and abnormal karyotypes were 36.2% (n = 17) and 63.8% (n = 30), respectively.

Of the patients without treatment, 71.1% (160/225) gave live birth (Table 2). The subsequent live birth rates were 70.0% and 71.9% in the patients with and without the risk allele of SNP5, respectively, and the respective percentages were 84.0% and 84.3% when cases with an abnormal embryonic karyotype were excluded. Among the 39 patients who received anticoagulant treatment, the subsequent live birth rats were 63.2% and 65.0% in the patients with and without the risk allele of SNP5, respectively. Treatment had no effect on the live birth rate in patients with the risk allele of SNP5.

According to the results of the univariate analysis using SNP5, there was no increase in the pregnancy loss rate associated with the presence of the SNP5 risk allele in the 190 patients without treatment or in chemical pregnancy or miscarriage caused by an abnormal embryonic karyotype (OR 1.027, 95% CI 0.463–2.276; P=.9487).

The results of multivariate analyses using SNP5, age, number of previous miscarriages, and presence of previous live births as variables revealed the absence of any influence of the SNP5 risk allele on the subsequent pregnancy loss rate (OR 1.187, 95% CI 0.506–2.784; P=.6941). The miscarriage rate in patients with a history of five to nine miscarriages was higher than that in the patients with a history of two miscarriages (OR 6.012, 95% CI 0.976–37.037; P=.0532).

TABLE 3

Subsequent live birth rate in subjects with and without the SNP5 risk allele.

	With risk allele T/G or G/G	Without risk allele T/T
Subsequent live birth rate in 225 patients without treatment	70.0% (63/90)	71.9% (97/135)
Subsequent live birth rate in 39 patients with treatment	63.2% (12/19)	65.0% (13/20)
Live birth rate after excluding cases with treatment or chemical pregnancy (n = 215)	73.3% (63/86)	75.2% (97/129)
Live birth rate after excluding cases with treatment or an abnormal embryonic karyotype (n = 190)	84.0% (63/75)	84.3% (97/115)

Note: SNP = single-nucleotide polymorphism.

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DISCUSSION

This case-control study demonstrated a significantly higher frequency of the minor alleles in the RPL group compared with the control group for SNP5 (P=.049), but not for SNPs 1–4 and 6. Existence of an association between SNP5 and RPL was originally reported by another Japanese group (17). Miyamura et al. examined patients with a history of three or more unexplained miscarriages (3.06 ± 0.3). The other inclusion and exclusion criteria were similar to those in the present study. It was a new finding that the dominant model should be selected, because the previous study did not perform max-statistics.

Earlier studies suggested that the M2 haplotype of the *ANXA5* gene promoter was significantly associated with the risk of RPL. Bogdanova et al. demonstrated for the first time that a sequence variation in the promoter region of the *ANXA5* gene represented a risk factor for RPL in a German population (16, 27). Another analysis of the significance of M2 strengthened the initial findings in Italian patients with RPL (28). Reporter gene assays showed that M2 reduced the in vitro activity of the *ANXA5* promoter to 37%–42% of normal (16). These data suggest that these SNPs are functional and can reduce the expression of *ANXA5*, thereby influencing the risk of RPL. A recent report showed that the M2 allele in heterozygous placentas resulted in a reduced expression level of the *ANXA5* mRNA by an average of 42% compared with the normal allele (29, 30). The M2 haplotype within the ANXA5 gene was also reported as a new thrombophilic risk factor during pregnancy (31). However, we could not discern any significant difference by max-statistics or haplotype analysis even though our present study included the largest number of subjects in both patient and control groups.

LD evaluation revealed that all except SNP5 manifested a strong LD (>0.95), consistent with the earlier report (17), suggesting that SNP5 may be an independent risk factor for RPL. The significance of SNP5 has not been analyzed in Western patients with RPL.

It has been suggested that annexin A5 molecules form an anticoagulation shield on the apical surface of the placental syncytiotrophoblasts, that may, in pregnancy, be disrupted by antiphospholipid antibodies (32, 33). In a mouse study,

infusion of anti-annexin A5 antibodies induced coagulation at the surface of the syncytiotrophoblast layer, leading to fetal loss, which indicates that annexin A5 protects the fetomaternal interface from the coagulation of maternal blood (34). The syncytiotrophoblasts that possess annexin A5 protein at their surface are of fetal origin and have a half-maternal and half-paternal genome. Tranquilli et al. proposed that carrier status of the fetus of thrombophilia such as factor V polymorphism should be considered to be risk factor for intrauterine fetal death (35). A recent report demonstrated that paternal and maternal carriage of the annexin A5 M2 haplotype were equal risk factors for RPL (36). Thus, it is still not clear whether the annexin A5 protein at the syncytiotrophoblast surface originates from the maternal circulation or is produced by the fetal syncytiotrophoblasts. Functional analysis of SNP5 or SNP5 genotyping of the fetus or placenta would be warranted to further clarify our findings.

A number of articles regarding association between SNPs and RPL have been published (27). An association between heritable thrombophilia, such as the factor V Leiden mutation, prothrombin mutation, and protein S deficiency, and RPL has been reported (9, 10), although the association is not well established (11).

The present study confirmed *ANXA5* SNP5 as a risk factor for RPL. However, the subsequent live birth rate was 84.0% and 84.3% in patients with and without the risk allele of SNP5, after the exclusion of cases with an abnormal embryonic karyotype, with no significant difference. This was the first study indicating the influence of *ANXA5* SNP5 on further pregnancy outcomes. The genome-wide association study proved that the effect of the one of many kinds of SNPs associated with a common disease is very small when the OR is relatively small (37). The *ANXA5* risk allele was not found to be a reliable clinical predictor of subsequent pregnancy outcome. Therefore, we propose that testing for this allele is not needed, because it is without clinical benefit and is an unnecessary expense.

The randomized controlled trial by Kaandrop et al. concluded that there was no effect of combined heparin and aspirin treatment for unexplained recurrent miscarriage (38). The study did not deny that anticoagulant therapy might be effective for a limited number of patients with miscarriage of unexplained cause, because abnormal embryonic karyotype was found to be the most common cause in cases with an unexplained etiology (6). Adequate selection of patients based on analysis of several kinds of SNPs might improve the therapeutic effectiveness. Further study is important to find SNPs with clinical predictive benefit associated with RPL.

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ORIGINAL RESEARCH

Cognitive behavior therapy for psychological distress in patients with recurrent miscarriage

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Objective: To examine the reduction of psychiatric symptoms using individual cognitive behavior therapy (CBT) for women who suffer from recurrent miscarriage (RM) and depression and/or anxiety.

Methods: Patients with RM and a score of five or higher for K6, a self-report screening scale for depression/anxiety, were interviewed to find information about stressful situations, thoughts, and consequent behaviors that are common and potential causes of psychological distress among RM patients. We then performed individual CBT on 14 patients with RM and depression/anxiety, referring to a list from the interviews, and examined the effects of CBT by a paired *t*-test.

Results: Fourteen women received CBT. The mean number of intervention times was 8.9 sessions (standard deviation [SD], 4.6 sessions). The average Beck Depression Inventory-Second Edition and State–Trait Anxiety Inventory–state anxiety scores, self-report screening scales for depression/anxiety, decreased from 13.6 (SD, 8.2) and 49.0 (SD, 7.1) at baseline to 5.2 (SD, 4.4) and 38.0 (SD, 10.2) posttherapy, respectively. These changes were statistically significant.

Conclusion: The current preliminary open study confirmed that individual CBT was potentially useful for women with RM and depression and/or anxiety. This finding is the first step towards creating a comprehensive psychological support system for women with RM.

Keywords: spontaneous abortion, psychological support, depression, anxiety

Introduction

Miscarriage is a very common complication in pregnancy, found in 15% of the women clinically identified as being pregnant. Recurrent miscarriage (RM), defined as two or more consecutive miscarriages, develops in about 5% of couples.[1] In recent years, RM has gained social recognition as a problem related to the decreasing birthrate.[2]

Causes of RM include abnormal chromosomes in either partner, existence of antiphospholipid antibodies, and uterine anomalies. Abnormal embryonic karyotype is also known as a cause, accounting for 25%–50% of miscarriages. However, more than half of RM cases remain unexplained.[1]

Miscarriage causes mental distress such as depression, anxiety, anger, and grief. If miscarriage is repeated, mental distress is sustained.[3] Craig et al[3] and Klock et al[4] reported that mental disorder was found in two-thirds of RM patients, and Craig et al[3] also reported that their anxiety level is similar to that of psychiatric outpatients. All of these suggest that RM patients are a group of people who need appropriate mental care.

In the infertility field, many studies concerning various psychological supports have been conducted for mental distress of patients. Their outcome is divided into two major kinds, improvement of psychological status and improvement of

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pregnancy rate. Three meta-analyses lead to a general conclusion that at least one of the two is to be expected.[5–7]

A few previous studies reported that some forms of psychological support including cognitive behavior therapy (CBT) for patients with single miscarriage were useful.[8–10] In the field of unexplained RM, it has been also indicated that psychological support such as counseling and increased regular checkups in the beginning pregnancy can help raise fertility rate,[11–13] but no further progress has been made. However, unlike for infertility patients or single-miscarriage patients, little research has been done for RM patients on the effects of psychological support with an outcome of reducing anxiety and depression in daily life.

CBT is one of the psychotherapies that is generally confirmed as being effective in reducing depression and/or anxiety.[14] In this study, we preliminarily examined the possibility of reducing depression and/or anxiety of the subjects through individual CBT as the first step for constructing a total psychological support system for RM. Generally, in each CBT session, the patient and the therapist utilize specific hardships in the patient's real life to collaboratively identify and examine her perspective (thoughts, recognition) and behavior. They then devise ways to control her distress or find coping strategies for her hardship. Accordingly, having information in advance about the kind of stressful situations, thoughts, and consequent behaviors which are common and potential causes of depression and/or anxiety among RM patients helps the therapist identify a problem to be discussed and in turn smoothly conduct a session. In addition, such information could be used in the course of developing a broadly available psychological support system in the future. Therefore, we first interviewed patients about what situations often cause them distress, and then conducted preliminary individual CBT sessions referring to the previous interview results and observed the change of depression and/or anxiety.

Materials and methods

Patients

Subjects were recruited from women who visited the specialized RM outpatient care at Nagoya City University Hospital during the period of April 2008 to September 2010; recruited subjects had a history of two or more consecutive miscarriages and had no children. Two weeks after systematic RM tests and explanation about the results, patients filled in K6,[15,16] a self-report questionnaire for depression and/or anxiety, and sent it to us by mail. Patients with a score of five or higher on the K6 were selected as subjects in this study.

With those subjects, a psychiatrist (YN) conducted Diagnostic and Statistical Manual of Mental Disorders (fourth edition, text revision) (DSM-IV-TR) diagnosis with Structured Clinical Interview for DSM-IV (SCID),[17] and the psychiatrist selected those who were diagnosed with mood disorder, anxiety disorder, or adjustment disorder caused by RM-related stress, pregnancy, or childbirth. Exclusion criteria included: (1) serious physical illnesses such as autoimmune disorder, cardiac disease, or chronic respiratory disease; (2) current or past psychotic disorder, bipolar disorder, cyclothymic disorder, eating disorder, developmental disorder, learning disorder, or substance dependence according to DSM-IV-TR; (3) current depression episode, dysthymic disorder, anxiety disorder, or adjustment disorder (by DSM-IV-TR) starting before the first miscarriage; (4) being pregnant at the time of the interview or beginning of CBT; and (5) previous CBT experience. As for new pregnancy during CBT, we planned to make a go/no-go decision when it happened because pregnancy may make some subjects physically (eg, morning sickness) and psychologically (eg, anxiety about miscarriage) unstable.

In general, CBT subjects were instructed to avoid pharmacotherapy. If they were already under pharmacotherapy, they were not allowed to switch medicines during CBT, except in cases of emergency. If a cause of RM was specified, its treatment had priority over CBT.

This study was approved by the Ethics Review Committee at Nagoya City University Graduate School of Medical Sciences. The subjects received explanation in writing and submitted informed consent before entering the study.

Procedure

Before starting psychological support by CBT, a researcher in this study (YN) with over 15 years of experience in clinical psychiatry and 10 years of experience as a CBT therapist interviewed a series of ten subjects in person, for 75–90 minutes each, and collected information about mental distress concerning pregnancy and children seen in their daily situations. Another psychiatrist (TF) with over 10 years of CBT experience who has been conducting research in psychosocial factors of RM, as well as an obstetrician (MS) who specializes in RM, then examined the interviews. By checking what was said (viewpoints, recognition) and how they behaved in stressful situations, as mentioned by subjects, several features, which seemed helpful in CBT, were extracted (Table 1).

The average age of the ten subjects interviewed was 32.3 (±3.5) years and the average number of miscarriages was

Table 1 Common thoughts and behaviors among women with recurrent miscarriage who are experiencing depression and/or anxiety

1. They believe that a woman who marries but does not have a baby is not a mature adult
2. They believe that people who have children are happy and that people who do not have children are unhappy
3. They have feelings of guilt that they have killed their own fetuses in uteri
4. They are afraid of both getting pregnant and of not getting pregnant
5. They are beleaguered with anxiety about the future and about how long their present situation will last
6. They are uncertain if each action in their daily life is good or bad for their next pregnancy
7. They miss many opportunities to enjoy themselves because they avoid places where there are many children and their parents, and participating in events where many mothers will be present
8. They avoid gatherings of relatives during the summer holidays or New Year holidays, or gatherings of peers such as class reunions
9. They and their spouses are likely to overlook women's fatigue and exhaustion
10. Not many women understand that their idea and their husband's idea are not the same regarding having a baby

2.7 (±0.9). No significant difference was found between these and the average age of 33.0 (standard deviation [SD] ± 4.8) and the average number of miscarriages of 2.7 (SD ± 1.0) of a series of 305 patients who visited our hospital for thorough examination. The causes of miscarriage for eight subjects were unexplained. Two suffered from uterine deformity, but they did not need surgery in order to become pregnant or to maintain pregnancy.[18] The average K6 score was 8.6 (±2.5). Those who were interviewed to extract common thoughts and behaviors continued regular treatment at the obstetrics department. They also received routine psychiatric outpatient therapy if they needed it.

After common thoughts and behaviors were obtained as indicated in Table 1, 14 subjects who met the eligibility criteria provided informed consent and entered this study between October 2008 and September 2010 (Figure 1).

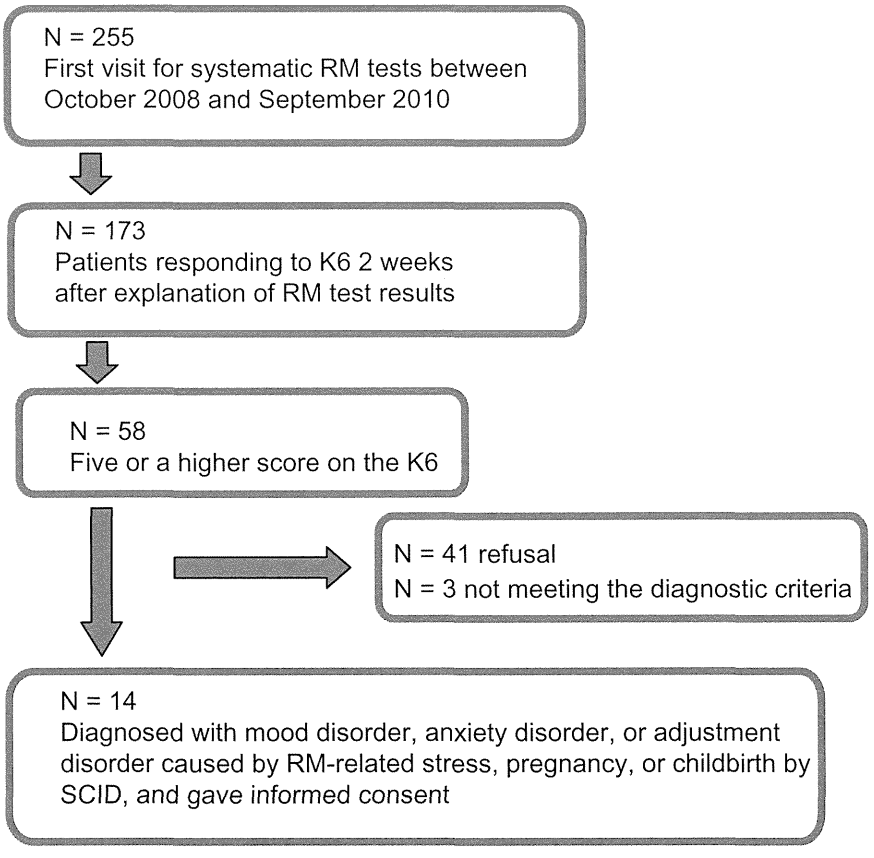

Figure 1 Patient flow chart for CBT.
Abbreviations: CBT, cognitive behavior therapy; N, number; RM, recurrent miscarriage; SCID, Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (fourth edition); K6, 6-item self-report instrument for screening for clinical depression or anxiety in adults.

The psychiatrist (YN) conducted CBT according to the individual treatment method developed by Beck[19] as well as by Beck et al (Table 1).[20] One session lasted approximately 50 minutes. The list in Table 1 was never shown to the patient since it was made as reference to help the therapist conduct smooth and effective sessions.

The basic rule for a course of sessions was that the sessions were to be held once a week, up to 16 times in total. However, in reality, flexible decisions had to be made depending on how profoundly distressed the patient was feeling in her daily life and how much the patient's depression and/or anxiety improved. In some cases, we allowed a patient to have a session once every 2 or more weeks after the 12th session.

Measure

K6

The K6 is a 6-item self-report instrument for screening for clinical depression or anxiety in adults.[15] The items cover how frequently the respondents experienced symptoms of psychological distress during the previous 30 days. The possible total scores range from 0 to 24. According to a Japanese validation study of the K6 in a general population, the positive predictive value for major depressive disorder or any anxiety disorder according to the DSM-IV-TR criteria was 0.49 for K6 scores of 10 or more.[16] The reliability and validity of the Japanese version has been previously confirmed.[16] The internal consistency of this instrument in the current sample was sufficient (Cronbach's $\alpha = 0.89$). In this study, women with a score of five or higher on the K6 result were included as subjects.

Beck Depression Inventory-Second Edition

The Beck Depression Inventory-Second Edition (BDI-II) is a 21-item self-report instrument for measuring the severity of depression in adolescents and adults.[21] The total scores range from 0 through 63. The severity of depression is categorized based on the following BDI-II scores in Japanese samples: a score of 13 or less is regarded as minimal (or remission); 14 to 19 is regarded as mild; 20 to 28 is regarded as moderate; and 29 or greater is regarded as severe. The reliability and validity of the Japanese version has been previously confirmed.[22] The internal consistency of this instrument in the current sample was sufficient (Cronbach's $\alpha = 0.92$).

State–Trait Anxiety Inventory–state anxiety

The State–Trait Anxiety Inventory–state (STAI-s) anxiety consists of 20 items and has a maximum score of 80.[23] State anxiety refers to the degree of anxiety at a particular point. Scores of over 50 indicate an extremely high level of anxiety (neurosis level). The reliability and validity of the Japanese version has been previously confirmed.[24] The internal consistency of this instrument in the current sample was sufficient (Cronbach's $\alpha = 0.91$).

BDI-II and STAI-s anxiety were adopted because they are both simple and useful scales to check the change in depression and/or anxiety severity. Following the usual CBT procedure, they were conducted right before each CBT session to assess the patient's condition.

Statistical analysis

Wilcoxon signed rank tests were conducted before and after CBT to see any statistically-significant change of BDI-II and STAI state anxiety. All analyses were performed using IBM SPSS Statistics, version 18 (IBM Corporation, Armonk, NY, USA). All statistical tests were two-tailed, and an alpha value of less than 0.05 was considered statistically significant.

Results

Fourteen patients, who met the criteria and consented, received CBT. The features of the samples are shown in Table 2. No significant difference was found between these and a series of 305 patients who visited our hospital for thorough examination in terms of the average age and number

Table 2 Sample characteristics: demographic and symptomatic data

	Mean	SD
Age (years)	34	4
Number of previous miscarriages	2.7	1.0
BDI-II before CBT	13.6	8.2
State anxiety in STAI before CBT	49	7.1
Number of CBT sessions	8.9	4.6
	%	Number
Education		
High school graduate	28.6	4
Junior college graduate	50.0	7
College graduate or higher	21.4	3
DSM-IV diagnosis		
Major depressive disorder	35.7	5
Adjustment disorder (with depressed mood)	35.7	5
Adjustment disorder (with mixed anxiety and depressed mood)	28.6	4
Adjustment disorder (with anxiety)	7.1	1
Specific phobia	7.1	1
Panic disorder	7.1	1
Posttraumatic stress disorders	7.1	1

Abbreviations: SD, standard deviation; BDI-II, Beck Depression Inventory-Second Edition; CBT, cognitive behavior therapy; STAI, The State–Trait Anxiety Inventory; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders (fourth edition).

of miscarriages. The causes of RM for nine of the patients (64.2%) were unexplained. Four patients had uterine deformity but their possibility of maintaining pregnancy was not expected to improve much by a surgery.[13] One patient was diagnosed with hyperthyroidism but her hormone value had been stable within the normal range for over 6 months under pharmacotherapy (desiccated thyroid 75 μg).

Two patients were diagnosed with moderate- to severe-level major depression disorder according to DSM-VI-TR; their BDI-II scores exceeded 30 points at baseline. The depression and/or anxiety severity of most of the patients was around the level of adjustment disorder according to DSM-IV-TR. One patient had been in pharmacotherapy and there was no change in her medication (75 mg/day sertraline, 1 mg/day lormetazepam) during CBT sessions. There was one patient who became pregnant in the 14th week of the 16 originally planned sessions. Since she complained of high anxiety about miscarriage and asked for extended treatment, a decision was made to have a session once a month until she reached a stable period, totaling 18 sessions.

Figure 2 shows the changes in BDI-II and STAI-s anxiety before and after CBT. STAI data for four patients were lost. The average BDI-II and STAI-s scores decreased from 13.6 (SD, 8.2) and 49.0 (SD, 7.1) at baseline to 5.2 (SD, 4.4) and 38.0 (SD, 10.2) at posttherapy, respectively. These changes were statistically significant (Wilcoxon signed rank test: BDI-II n = 14, z = −3.2, $P = 0.001$; STAI-s n = 10, z = −2.4, $P = 0.016$).

Discussion

This is the first attempt of psychological support with CBT for patients with RM. This study preliminarily confirmed that CBT, in concert with references to the list of common hardships prepared in advance, can decrease depression and/or anxiety of patients with RM. It is appropriate to say that, based on the SCID diagnosis and the average BDI-II scores at baseline and posttreatment, the mild depressive state of the 14 subjects in this study recovered to normal levels. It can be also said from the average scores of STAI-s anxiety at baseline and at posttreatment that the subjects came out of their previous condition at a mildly overanxious level.

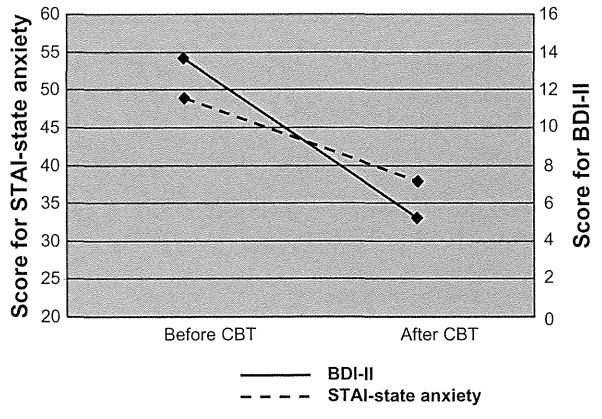

Figure 2 Comparison of BDI-II and STAI before and after CBT.
Abbreviations: BDI-II, Beck Depression Inventory-Second Edition; STAI, State–Trait Anxiety Inventory; CBT, cognitive behavior therapy.

It has been already mentioned that the childbirth rate of RM patients with an unexplained cause may be raised by increasing the number of checkups and providing counseling in early pregnancy.[8–10] Additionally, we have already shown that depression raises the possibility of miscarriage.[25] All these factors indicate that recovery from depression may lead to an increased childbirth rate. Therefore, although we were unable to examine successful birthrate this time, we should, and certainly hope to, have childbirth rate as an outcome in our future research.

However, there are several limitations in this study. First, it was an open label study with no control group. There exists a possibility that depression and/or anxiety decreased as a natural course. Second, it was left unexamined whether the positive effect could be maintained after the end of treatment, despite the fact continuing improvement of depression and/or anxiety in the life of patients is the most important outcome. Moreover, since there were only two estimating points, one at the start and another at the end of CBT sessions, the process of improvement could not be discussed. The third limitation is that the strict procedure of qualitative research, such as a qualitative and descriptive research method, was not taken in constructing Table 1. However, it was designed as reference data to conduct CBT smoothly in the first place, and in fact, Table 1 fulfilled the purpose. For example, more than half of the subjects covered items 1, 3, 5, 8, and 10 in their sessions.

We routinely hand out a form to patients to write comments freely when they visit our hospital for thorough examination. Although they often mention experiencing various kinds of psychological distress, only a few patients actually come for individual CBT. As Boivin et al[26] pointed out, it is likely that patients with mild psychological distress do not feel a need to depend on a specialist, while those experiencing severe distress do not reach a point where they want to meet with CBT therapists, or any other specialist engaging in psychological support, because these individuals worry about how much these services may cost and what the specialist may be like. Moreover, many patients might naturally be resistant to self-disclosure.

Recently, there have been many attempts concerning CBT on the Internet.[27,28] The approach using the Internet may have advantages because it can be accessed from anywhere at low cost, requires less self-disclosure, calls for less concern about compatibility with a therapist, and therefore, seems easier to start. Upon creating online CBT content in the future, the information from Table 1 will contribute considerably.

On the other hand, Wischmann[29] recommended that face-to-face consultations be used for complicated matters for infertility patients' mental distress. Therefore, for RM patients who are suffering from depression and/or anxiety, we plan to recommend a psychological support program based on CBT via the Web before inviting patients to engage in individual CBT. Once such a system takes off, we hope to conduct a randomized controlled trial with birthrate, as well as depression and/or anxiety, as study endpoints.

This pilot study, which preliminarily indicated a decrease in depression and/or anxiety for RM patients by individual CBT, was the first step towards creating a comprehensive psychological support system for RM.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work. The authors disclosed no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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Peripheral natural killer cell activity as a predictor of recurrent pregnancy loss: a large cohort study

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Objective: To determine the predictive value of preconceptional peripheral blood natural killer (pNK) cell activity in patients with recurrent pregnancy loss (RPL).
Design: Cohort study.
Setting: University department.
Patient(s): A total of 552 patients with a history of two to six consecutive miscarriages.
Intervention(s): None.
Main Outcome Measure(s): The predictive value of preconceptional pNK cell activity for subsequent miscarriage was analyzed using multivariable logistic regression analysis, with age, number of previous miscarriages, and presence/absence of previous live births and bed rest as covariates.
Result(s): Age and number of previous miscarriages, but not high pNK cell activity, were found to be independent risk factors for a subsequent miscarriage. No effect of bed rest and previous live birth on the likelihood of live birth was observed (odds ratios 1.28 [95% confidence interval 0.81–2.02] and 0.91 [0.52–1.59], respectively).
Conclusion(s): Elevated pNK cell activity was found to not be an independent risk factor for subsequent miscarriage. Clinicians should not measure the plasma NK activity as a systematic recurrent pregnancy loss examination, because its clinical significance is yet to be established. (Fertil Steril® 2013;100:1629–34. ©2013 by American Society for Reproductive Medicine.)
Key Words: Recurrent pregnancy loss, natural killer cell activity, predictor, cohort study

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Established causes of recurrent pregnancy loss (RPL) include presence of antiphospholipid antibodies in the serum, presence of uterine anomalies, and presence of abnormal chromosomes, particularly translocations, in either partner (1–3). According to previous reports, in approximately half of the cases seen at research centers, the cause of RPL remains unexplained despite conventional examinations conducted to identify the cause (4–6).

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Cytotrophoblasts that express human leukocyte antigen G (HLA-G) come in direct contact with maternal lymphocytes. Many natural killer (NK)-like large granular lymphocytes have been detected in the human decidua of early pregnancy (7). Large numbers of NK cells appear in the mid-secretory phase. Natural killer cells have been thought to play a key role in the establishment of successful pregnancy by facilitating immunologic adaptation of the semiallogenic developing embryo. Recently, Fu et al. (8) reported that recruitment of TH17 cells and local inflammation can occur at the maternal–fetal interface during natural allogenic pregnancies, and that decidual NK cells promote immune tolerance and successful pregnancy

by diminishing the recruitment of inflammatory TH17 cells via interferon-γ secreted by the $CD56^{bright}CD27^{+}$ NK cell subset. This NK cell–mediated regulatory response is lost in patients with recurrent spontaneous abortions, resulting in a prominent TH17 response and extensive local inflammation.

We previously reported (9) that elevated preconceptional peripheral blood NK (pNK) cell activity may be predictive of subsequent miscarriage in 68 patients with RPL. The 24 women with high pNK cell activity, defined as a value equal to or exceeding the mean pNK cell activity of the 47 controls + 1 SD, had a significantly higher miscarriage rate in the subsequent pregnancy than the 44 women with normal pNK cell activity (71% vs. 20%; relative risk 3.5; 95% confidence interval [CI] 1.8–6.5). However, some studies have shown no differences in pNK cell parameters between patients with RPL and controls (10–12). Whereas 90% of pNK cells are $CD56^{dim}$ and $CD16^{+}$, 80% of the uterine NK cells are $CD56^{bright}$ and $CD16^{-}$ (13, 14). Peripheral blood NK cells are phenotypically and functionally different from uterine NK cells. Decidual leukocytes have low cytotoxic activity as compared with peripheral lymphocytes (15).

The sample size of our previous study was too small. The prognostic value of measuring pNK cell parameters remains uncertain (16). Therefore, we conducted a cohort study of 552 patients with RPL to determine whether pNK might indeed be predictive of subsequent miscarriage.

MATERIALS AND METHODS

Patients

We studied 1,127 patients with a history of two or more (2 to 12) consecutive miscarriages, in whom the study examinations could be completed and subsequent pregnancies were established between January 1996 and May 2011 in Nagoya City University Hospital. Patients with identifiable causes and patients who received any kinds of treatment were excluded from the present cohort.

The conventional examinations were completed in all patients, including hysterosalpingography, transvaginal ultrasonography, chromosomal analysis of both partners, determination of the presence/absence of antiphospholipid antibodies, including lupus anticoagulant (LA), by diluted activated partial thromboplastin time (aPTT), diluted Russel viper venom time (RVVT), and β2 glycoprotein I–dependent anticardiolipin antibody methods (17), and blood tests for hypothyroidism and diabetes mellitus, before the subsequent pregnancy.

Antiphospholipid antibody syndrome (APS) was diagnosed according to the criteria of the International Congress on Antiphospholipid Antibodies (18). Patients with APS were treated with low-dose aspirin plus heparin (19). Diabetes mellitus, hyper- or hypothyroidism, and hyperprolactinemia in the patients were controlled with medication before conception. Patients with three or more unexplained miscarriages received paternal mononuclear cell immunization from 1996 to 1999, a biologic response modifier from 1996 to 2004 (20), and low-dose aspirin and/or heparin from 2000 to 2007.

Gestational age was calculated from basal body temperature charts. A total of 654 patients were admitted for rest, and ultrasonography was performed twice per week from 4 to 8 weeks of gestation before 2004. The pregnancy outcome of 473 patients was followed once per week by ultrasonography without admission after 2004.

The study was conducted with the approval of the Research Ethics Committee at Nagoya City University Medical School.

Measurement

Preconceptional pNK cell activity was examined in the midsecretory phase. Peripheral blood NK cell activity was measured by a chromium-51 release cytotoxicity assay, with K562 human myeloid leukemia cells as the targets. A total of 3.7×10^3 Bq ^{51}Cr-labeled target cells (1×10^4 per well) were seeded with 2×10^5 effector cells per well (fresh peripheral blood mononuclear cells) in triplicate, in U-bottomed 96-well plates. After 4-hour incubation at 37°C, the activity in the supernatant from each well was measured in an autogramma scintillation counter. The percentage cytotoxicity was calculated as follows: ([test cpm−spontaneous cpm]/[maximum cpm−spontaneous cpm]) × 100, where cpm = counts per minute.

Lupus anticoagulant was detected using fivefold diluted aPTT methods, as previously described, with brain cephalin (Automated aPTT; Organon Teknica) as the phospholipid reagent (17). The 1:1 mixing test was performed at the same time. The clotting time was measured using an Option 4 Biomerieux calculator. Lupus anticoagulant was considered positive when prolonged clotting times (>mean + 3 SD of 104 healthy nonpregnant control plasma, 7.37 seconds) failed to correct when mixed 1:1 with standard plasma. Diluted Russel viper venom time for LA was performed as previously described (Gradipore). To detect β2 glycoprotein I–dependent anticardiolipin antibody, we used a modified ELISA system (Yamasa).

Analysis

Patients with identifiable causes and patients who received any kinds of treatments were excluded from the present cohort. Biochemical pregnancy, ectopic pregnancy, and hydatidiform mole were excluded from the analysis of the subsequent pregnancy outcome.

Miscarriage rate was analyzed according to pNK cell activity, age, number of previous miscarriages, and presence/absence of previous live births and bed rest. Peripheral blood NK cells, age, and previous number of miscarriages were categorized into quartiles, because they showed normal distribution. The previous number of miscarriages was categorized into two, three, four, or five to six.

Crude logistic regression was performed to examine the predictive value of pNK cell activity for subsequent miscarriage. We also examined the influence of age, previous number of miscarriages, and presence/absence of previous live birth and bed rest on the likelihood of subsequent miscarriage.

Age is well known to influence the miscarriage rate. Age is also associated with number of previous miscarriages and number of previous live births. Thus, first, we chose

age-adjusted logistic regression. This analysis was applied to all the variables listed in the table except age.

Furthermore, multivariable logistic regression analysis was performed using pNK cell activity, age, number of previous miscarriages, and presence/absence of previous live births and bed rest as covariates. Linear multivariable logistic regression analysis was also performed using pNK cell activity, age, and number of previous miscarriages.

The analysis was carried out using SAS version 19.0 (SAS Institute), and $P<.05$ was considered to denote statistical significance.

RESULTS

In the subjected 1,127 patients, 4.4% (50) had an abnormal chromosome in either partner, 4.1% (46) of patients had a major uterine anomaly, 3.4% (38) had thyroid disease, 1.9% (21) had diabetes mellitus, and 2.9% (33) had APS (Fig. 1). In total, 180 patients were excluded from the cohort because several patients had two or three identifiable causes.

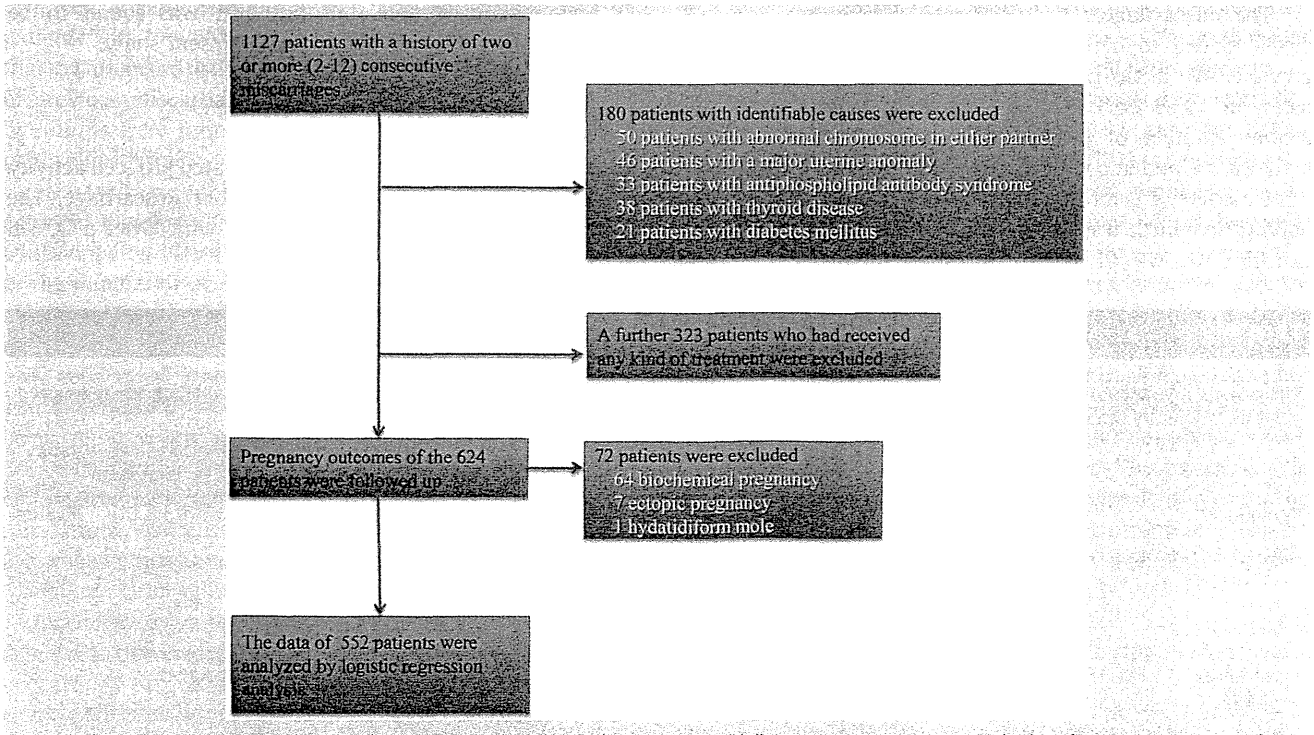
To eliminate the influence of the treatment, a further 323 patients who received any kind of treatment were excluded. A total of 72 patients—64 biochemical pregnancy, 7 ectopic pregnancy, and 1 hydatidiform mole—were excluded in the present study (Fig. 1).

The miscarriage rate of a total of 552 patients with unexplained RPL who received no medication is shown in Table 1. The subsequent miscarriage rate was 22.5% (124 of 552). The mean (SD) age and median (interquartile range) number of previous miscarriages were 31.9 (4.37) and 2 (2–3), respectively. The live birth rates of patients with previous two, three, four, five, and six miscarriages were 81.1% (309 of 381), 71.2% (99 of 139), 65.4% (17 of 26), 60.0% (3 of 5), and 0 (0 of 1), respectively.

Linear multivariable logistic regression showed that pNK was not an independent risk factor for subsequent miscarriage. However, in the crude analysis of the categorization of each variable, the miscarriage rate in the patients with 5%–24% pNK cell activity was significantly higher than that in the patients with 25%–34% pNK cell activity ($P=.046$). On the other hand, the miscarriage rate in the patients with 47%–78% pNK cell activity was similar to that in the patients with 25%–34% pNK cell activity. The plasma NK cell activity showed a weak inverse correlation with age in the 1,127 patients ($r = -0.068$).

Five variables, namely pNK cell activity, age, number of previous miscarriages, and absence of bed rest and previous live birth, were entered into the multiple logistic regression analysis for subsequent miscarriage detection in all 552 patients. The miscarriage rate in patients with 25%–34% pNK cell activity tended to be higher than that in patients with 5%–24% pNK cell activity (odds ratio [OR] 0.56, 95% confidence interval [CI] 0.31–1.00, $P=.051$). Crude, age-adjusted, and multivariable logistic regression analyses showed similar results in relation to pNK cell activity. Elevated pNK cell activity was confirmed to not be an independent risk factor for a subsequent miscarriage.

FIGURE 1



A total of 552 patients were analyzed in the present study. Of the 1,127 women initially enrolled, 180 patients with identifiable causes, 323 patients who received any kind of medication, and 72 patients whose pregnancy outcomes were biochemical or ectopic pregnancy were excluded.

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TABLE 1

Miscarriage rate according to pNK cell activity, age, and number of previous miscarriages, and age-adjusted and multivariable logistic regression analysis to identify the risk factors for subsequent miscarriage.

		Crude analysis logistic regression		Age-adjusted logistic regression[a]		Multivariable logistic regression[b]		
Parameter	Miscarriage rate, % (n)	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Trend *P* value
Peripheral NK cell activity (%)								.365
5–24	28.1 (41/146)	Reference		Reference		Reference		
25–34	17.9 (24/134)	0.56 (0.32–1.00)	.046	0.55 (0.31–0.98)	.042	0.56 (0.31–1.00)	.051	
35–46	22.6 (31/137)	0.75 (0.44–1.28)	.293	0.73 (0.42–1.26)	.261	0.78 (0.45–1.36)	.385	
47–74	20.7 (28/135)	0.67 (0.39–1.16)	.154	0.69 (0.39–1.20)	.186	0.73 (0.41–1.30)	.282	
Age (y)								.0002
19–29	13.2 (22/167)	Reference				Reference		
30–31	28.7 (33/115)	2.65 (1.45–4.85)	.0015			2.49 (1.35–4.59)	.0036	
32–35	20.9 (33/158)	1.74 (0.96–3.14)	.0658			1.46 (0.79–2.71)	.226	
36–45	32.1 (36/112)	3.12 (1.72–5.68)	.0002			2.54 (1.35–4.76)	.0037	
No. of previous miscarriages								.0014
2	18.9 (72/381)	Reference		Reference		Reference		
3	28.8 (40/139)	1.73 (1.11–2.71)	.012	1.57 (0.99–2.48)	.055	1.38 (0.85–2.26)	.198	
4	34.6 (9/26)	2.27 (0.97–5.30)	.052	1.84 (0.77–4.37)	.168	1.65 (0.67–1.10)	.280	
5–6	50.0 (3/6)	4.29 (0.85–21.70)	.090	3.56 (0.68–18.55)	.132	3.73 (0.69–20.10)	.126	

[a] The only covariate used was age for the age-adjusted logistic regression analysis. This analysis was applied to all the variables listed in the table except age.
[b] The covariates used for the multivariable logistic regression analysis were pNK activity, age, number of previous miscarriages, presence/absence of previous live births, and presence/absence of bed rest.

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The miscarriage rate in the patients without bed rest was significantly higher than that in the patients who were admitted for rest ($P=.016$; Table 2). However, there were differences in the mean [SD] age (30.9 [4.0] vs. 32.8 [4.5] years; $P<.0001$) and the median number of previous miscarriages (three vs. two) between the patients with and without bed rest.

The miscarriage rate in patients without previous live births tended to be lower than that in the patients with previous live births ($P=.096$). There were differences in the mean age (33.9 [3.7] vs. 31.6 [4.4] years; $P<.0001$) and the median number of previous miscarriages (three vs. two) between the patients with and without previous live births.

No effect of bed rest and previous live birth on the likelihood of live birth was observed (OR 1.28, 95% CI 0.81–2.02 and OR 0.91, 95% CI 0.52–1.59, respectively).

Age and number of previous miscarriages were determined to be risk factors for subsequent miscarriage according to both crude and linear multivariable logistic regression.

Age and numbers of previous miscarriages were confirmed to be independent risk factors. However, number of previous miscarriage, but not age, was found to be influenced by the other factors in the present study.

DISCUSSION

The results of this study suggest that elevated pNK cell activity is not a reliable predictor of subsequent miscarriage. The miscarriage rate was higher in patients with lower pNK cell activity.

TABLE 2

Miscarriage rate according to the presence/absence of previous live births and bed rest, and age-adjusted and multivariable logistic regression analysis to identify the risk factors for subsequent miscarriage.

				Crude analysis logistic regression		Age-adjusted logistic regression[a]		Multivariable logistic regression[b]	
Parameter	Miscarriage rate, % (n)	Mean (SD) age (y)	Median no. of previous miscarriage	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Absence of previous live birth									
Presence	28.4 (25/88)*	33.9 (3.7)*	3*	Reference		Reference		Reference	
Absence	21.3 (99/464)*	31.6 (4.4)*	2*	0.68 (0.41–1.14)	.096	0.68 (0.41–1.14)	.146	0.91 (0.52–1.59)	.736
Absence of bed rest									
Presence	18.2 (47/258)*	30.9 (4.0)*	3*	Reference		Reference		Reference	
Absence	26.2 (77/294)*	32.8 (4.5)*	2*	1.59 (1.06–2.40)	.016	1.59 (1.06–2)	.026	1.28 (0.81–2.02)	.288

[a] The only covariate used was age for the age-adjusted logistic regression analysis. This analysis was applied to all the variables listed in the table except age.
[b] The covariates used for the multivariable logistic regression analysis were pNK activity, age, number of previous miscarriages, presence/absence of previous live births, and presence/absence of bed rest.
* $P < .05$ was considered to denote statistical significance.

Katano. Pregnancy loss and natural killer. Fertil Steril 2013.

Uterine endometrial NK (uNK) cell activity is known to be strongly involved in the maintenance of normal pregnancy. Lachapelle et al. (21) proved that the proportion of uNK cells was identical in recurrent miscarriage (RM) patients and normal controls, but the $CD56^{bright}$ and $CD16^{-}$ NK cell subset, which is predominant in normal decidua and endometrium, was significantly decreased in favor of an important contingent of $CD56^{dim}$ and $CD16^{+}$ NK cells in all patients. Quenby et al. (22) demonstrated that prednisolone therapy during the first trimester of pregnancy reduced the risk of miscarriages and improved the live birth rate in patients with idiopathic RM and increased the numbers of uNK cells in the endometrium. Measurement of pNK cell activity has been performed to determine whether it might be predictive of a successful subsequent pregnancy (23, 24). We have reported for the first time that elevated pNK cell activity might be predictive of subsequent miscarriage in patients with RM (9). Some have affirmed, whereas others have denied, the predictive value of pNK for the subsequent pregnancy outcome. However, none of these reports were based on studies of large cohorts, and there is no clear evidence yet (16, 25).

Patients with unexplained RM have been treated empirically with expensive immunoglobulin, on the basis of the conjecture that the functions of uNK cells and pNK cells are similar and that, therefore, measurement of pNK cell activity would reflect uNK cell activity. Tang et al. (16) reported a systematic review and came to the conclusion that there is no association between the subsequent pregnancy outcome and either pNK or uNK cell activity in women with RM and infertility. In the present study the correlation between the subsequent pregnancy outcome and pNK cell activity was not linear. The miscarriage rate in patients with low pNK cell activity tended to be higher than that in patients with 25%–74% pNK cell activity. Age, number of previous miscarriages, bed rest, and number of previous live births were found to exert no significant influence on pNK cell activity.

It is well known that stress and exercise increase pNK cell activity; therefore, these factors should be borne in mind while drawing blood for testing. Abnormal data pertaining to the number or activity of pNK cells may reflect transient stress reactions in daily life. It is not clear whether uNK cells may have the same significance. Peripheral blood NK and uNK cells are different types of cells, and both the models and functions of these cells are entirely different. It has been reported that measurement of pNK cell activity does not provide any information on the condition of the endometrial membrane (7, 14, 25). There is also no evidence of treatment using the data on pNK cell activity. We do not recommend measurement of pNK cell activity as part of the systematic examination in patients with RPL.

Mentally depressed patients with RPL need tender loving care (26). However, there is no evidence that subsequent miscarriage can be prevented by hospitalization. Klebanoff et al. (27) concluded that there was no difference in the miscarriage rate between women who had a heavy workload and long working hours and wives of male residents who had many kinds of jobs. Duckitt et al. (28) found no direct evidence from randomized, controlled trials regarding the influence of bed rest in women with unexplained RM. In the present study the live birth rate in patients without bed rest was significantly lower than that in the patients who were admitted for rest. However, there were significant differences in the mean age and median number of previous miscarriages between the patients with and without bed rest, because the average age of women at pregnancy is increasing year by year in Japan. Neither age-adjusted nor multivariable logistic regression analysis showed any effect of bed rest on the live birth rate. We concluded that there is no necessity to advise preventive bed rest for pregnant women, in the absence of symptoms of threatened abortion.

This study revealed that previous live birth was not predictive of a subsequent live birth, although there have been a few reports suggesting a favorable influence of a previous live birth in secondary RM patients (29, 30). Nielsen (31) reported that secondary RM is more common after the birth of a boy and that the subsequent live birth rate is reduced in secondary RM patients with a firstborn boy, owing to the pathogenic role of the aberrant maternal H-Y immune response. Both our previous study and the crude analysis in the present study indicated that the live birth rate increased as the number of previous miscarriages increased (32). However, the significant difference disappeared after adjustment for age, because age also increased with increasing number of previous miscarriages.

In more than half of the cases, the cause of RPL remains unexplained despite conventional examinations (4, 5). Recently we found that an abnormal embryonic karyotype was the most frequent cause, accounting for as much as 41% of the cases, and the percentage of truly unexplained was limited to 24.5% (33). Associations have been reported between many kinds of polymorphisms, such as those of annexin A5 and NLRP7, and RPL (34, 35). The influence of one single-nucleotide polymorphism associated with RPL might be speculated to be very small, because the OR of each gene mutation is relatively small (34). Even though it would be highly desirable, it might be difficult to identify clinically useful predictors of the outcome of a subsequent pregnancy.

We previously reported that elevated pNK cell activity may be predictive of subsequent miscarriage in patients with RPL (9). However, we wish to correct our initial conclusion, because in this study, high pNK cell activity was confirmed to not be an independent risk factor for subsequent miscarriage. Clinicians should not measure pNK activity as a systematic RPL examination, because the clinical significance or treatment method is yet to be established. Patients need not give up working, because no effect of bed rest on the likelihood of live birth was observed.

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Müllerian anomalies and recurrent miscarriage

Mayumi Sugiura-Ogasawara, Yasuhiko Ozaki, and Nobuhiro Suzumori

Purpose of review
To review the prevalence of congenital uterine anomalies and pregnancy outcomes in patients with these anomalies.

Recent findings
Women with a history of recurrent miscarriage have been estimated to have a 3.2–10.4% likelihood of having a major uterine anomaly except arcuate uterus. Hysterosalpingography and/or 2D ultrasound can be used as the initial screening tools. The American Fertility Society classification of Müllerian anomalies is the most commonly utilized standardized classification. However, there is still no international consensus to distinguish between septate and bicornuate uteri. A total of 35.1–65.9% of patients with bicornuate or septate uteri give live births after correctional surgery. In regard to the live birth rate in the absence of surgery, it has been reported that 33.3–59.5% of patients with such anomalies had a successful first pregnancy after the examination, as compared to 71.7% of individuals with normal uteri ($P=0.084$), with no significant difference in the cumulative live birth rate (78.0 and 85.5%, respectively) between the two groups.

Summary
Randomized controlled trials comparing the pregnancy outcomes between cases treated and not treated by surgery among patients with a history of recurrent miscarriage are needed because it is not established whether surgery could improve live birth rate.

Keywords
bicornuate uterus, congenital uterine anomaly, recurrent miscarriage, septate uterus

INTRODUCTION

Uterine development involves three main stages, including development of both Müllerian ducts, fusion of the two ducts, and septal absorption [1]. Congenital uterine anomalies may arise from malformations at any step of the Müllerian developmental process. Recently, a novel mutation of *HOXA10* gene that affected the transcriptional regulation capacity was found in one of 109 patients with uterine anomalies [2]. Several kinds of gene mutations may contribute to the development of uterine anomalies.

Uterine anomalies complicated with obstructed hemivagina can be diagnosed in young women because of severe dysmenorrhea and pelvic pain. A new variety of Müllerian anomalies, the accessory and cavitated uterine masses, was reported to raise awareness because they have been reported as juvenile cystic adenomyoma [3▪].

Uterine anomalies are associated with a high rate of recurrent miscarriage with normal embryonic karyotype [4]. A recent cohort study indicated that the presence of an anomaly was associated with higher rates of preterm birth less than 34 weeks [adjusted odds ratio (OR), 7.4; 95% confidence interval (CI), 4.8–11.4; $P<0.01$], preterm birth less than 37 weeks (OR, 5.9; 95% CI, 4.3–8.1; $P<0.01$), primary nonbreech cesarean delivery (OR, 2.6; 95% CI, 1.7–4.0; $P<0.01$), preterm premature rupture of membranes (OR, 3.2; 95% CI, 1.8–5.6; $P<0.01$), and breech presentation (OR, 8.6; 95% CI, 6.2–12.0; $P<0.01$) [5].

The American Fertility Society (AFS) classification of Müllerian anomalies is currently considered as the standard classification [6], although it has the limitation that it does not specify the diagnostic methods or criteria, because no objective methods that must be used to classify the anomalies are described and the classification is based on the subjective impressions of the clinician performing the tests.

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KEY POINTS

- Congenital uterine anomalies have a negative impact on reproductive outcome in couples with recurrent miscarriage and are associated with further miscarriage with a normal embryonic karyotype.
- Surgery did not seem to have any beneficial effect in terms of increasing the live birth rate as compared to the live birth rates reported without surgery in the literature.
- An RCT comparing the live birth rate with and without surgery is needed.

Saravelos *et al.* [7] concluded from a systematic review that the most accurate diagnostic procedures are a combination of hysteroscopy and laparoscopy, sonohysterography (SHG) and three-dimensional (3D) transvaginal ultrasound. From another systematic review, Chan *et al.* [8] included 3D ultrasound, laparoscopy or laparotomy performed in conjunction with hysteroscopy or hysterosalpingography (HSG), MRI, and saline SHG as the 'optimal diagnostic tests'. Chan *et al.* also described that the prevalence of uterine anomalies in various populations diagnosed by suboptimal tests was consistent with that in populations in which the diagnosis was based on optimal tests. HSG and/or 2D ultrasound can be used as the initial screening tools, and combined hysteroscopy and laparoscopy, SHG and 3D ultrasound can be used to arrive at a definitive diagnosis.

DIAGNOSTIC CRITERIA FOR CONGENITAL UTERINE ANOMALIES

HSG has been the most frequently used diagnostic modality for the diagnosis of congenital anomalies [6]. However, when used alone, it does not allow a septate uterus to be distinguished from a bicornuate uterus. Acien described that if careful observation of the external shape of the uterus at laparoscopy reveals any visible depression in the middle part of the fundic uterine wall associated with an overall widening, bicornuate uterus can be diagnosed [9]. Laparoscopy/HSG, which allows examination of both the uterine cavity and the external uterine contour, has hitherto been needed to precisely ascertain the type of anomaly in these patients, in accordance with the AFS classification of Müllerian anomalies.

Tompkin's Index (the height of the defect/length of the interostia line) has been used to distinguish between arcuate uterus and a mild septate or bicornuate uterus [10]. A Tompkin's Index more than 25% is considered to indicate a septate or bicornuate uterus. It has also been reported that an angle less than 75° between the uterine horns is suggestive of a septate uterus, whereas an angle greater than105° is indicative of a bicornuate uterus [1].

Different authors implement their own criteria. Fedele *et al.* [11] and Troiano and McCarthy [12] considered a uterus to be septate rather than bicornuate, in the presence of a fundal distal border indentation of 5 mm or less above the line joining the two ostia (interostial line). Wu *et al.* [13] and Woelfer *et al.* [14] provided new 3D ultrasound criteria, and indicated that a bicornuate uterus can be distinguished from a septate uterus when the fundal indentation dividing the two cornua was more than 10 mm in depth. These criteria are useful for making a decision on transcervical resection for the septum. However, the criteria differ from those of the AFS or Acien criteria. The prevalence of a bicornuate uterus may be higher according to the AFS or Acien classical criteria, and that of a septate uterus may be higher according to the new criteria.

The absence of an established classification system is a drawback that needs to be addressed urgently. International consensus for distinction between bicornuate and septate uteri should be established urgently.

PREVALENCE OF MAJOR UTERINE ANOMALIES

The frequency of congenital uterine anomalies has been reported to vary between 1.8 and 37.6% in women with a history of recurrent miscarriage, the variation largely depending on the methods and the criteria selected for the diagnoses [15–19].

Chan *et al.* conducted a systematic review of 94 studies with 86 861 women. 'Optimal diagnostic tests' had been used in 41 of 94 studies. Overall, 5.5% (95% CI, 3.5–8.5) of the unselected population were shown to have a uterine anomaly diagnosed by an optimal test. The prevalence of uterine anomalies was not higher in women with infertility (8.0%; 95% CI, 5.3–12.0, $P=0.239$) as compared with that in an unselected population. The incidence of uterine anomalies was found to be significantly higher in women with a history of miscarriage (13.3%; 95% CI, 8.9–20; $P=0.011$) and miscarriage in association with infertility (24.5%; 95% CI, 18.3–32.8; $P<0.001$) than in the unselected population. The prevalence of anomalies in women with two or more miscarriages (10.9%; 95% CI, 3.6–33.3) was not significantly different from that in women with three or more miscarriages (15.4%, 95% CI, 10.3–23, $P=0.572$).

The prevalence of arcuate uterus, as diagnosed by optimal tests, was not increased in infertile women (1.8%; 95% CI, 0.8–4.1) or in women with a history of miscarriage (2.9%; 95% CI, 0.9–9.6), as compared with that in an unselected population (3.9%; 95% CI, 2.1–7.1). Arcuate uterus seems to be a normal variant and not a uterine anomaly. According to the systematic review carried out by Chan *et al.*, the prevalence in women with miscarriage was 10.4%.

Major malformations such as septate, bicornuate or unicornuate uterus, and uterus didelphys were found in 3.2% of the 1676 patients in our previous study, based on the AFS and Acien criteria [4].

Saravelos *et al.* [7] concluded, based on hysteroscopy, SHG, 3D ultrasound and laparoscopy, that the prevalence of congenital uterine anomalies was 2.4% in the general population, 5.6% in women with infertility, and 7.1% in women with recurrent miscarriages. Thus, women with recurrent miscarriages have a 3.2–10.4% likelihood of having a major uterine anomaly.

Identifiable causes of recurrent miscarriage include antiphospholipid antibody syndrome, uterine anomalies, and chromosomal abnormalities, particularly translocations, in either partner [20]. According to previous reports, in about half of the cases seen at research centers, the cause of recurrent miscarriage remains unexplained despite conventional examinations conducted to identify the cause [17,21]. However, in our recent study including 482 patients in whom both chromosomal analysis of the aborted conceptus and conventional examination were undertaken, 41% of recurrent miscarriages were found to be caused by abnormal embryonic karyotype [22▪]. Uterine anomalies were rare in cases of secondary recurrent miscarriage and women above 40 years of age. Thus, the prevalence of uterine anomalies depends on characteristics such as the age and number of previous miscarriages.

SURGERY

Affected patients have been offered surgery in an attempt to restore the uterine anatomy. The live birth rates after surgery in studies including a relative large number of patients are summarized in Table 1 [4,16,23–30]. Makino *et al.* [16] described that 84.8% (39/46) of the postoperative pregnancies could be successfully carried through to full term. However, the outcomes of the remaining 25 patients who underwent metroplasty remain unclear. Thus, the live birth rate per patient was 54.9%.

Candiani *et al.* [24] reported that 68% (45/66) of patients with a septate uterus and 76% (50/66) of

Table 1. Live birth rate with and without surgery in patients with congenital uterine anomalies

	Surgery						No surgery		
	Makino *et al.* [16]	Candiani *et al.* [24]	Ayhan *et al.* [25]	DeCherney *et al.* [26]	Daly *et al.* [27]	Lee and Hickok [28]	Kormanyos *et al.* [29]	Sugiura-Ogasawara *et al.* [4]	Ghi *et al.* [32▪]
No. of patients	71	144	89	103	55	40	94	42	24
Type of anomaly	Arcuate, septate	73 septate, 71 bicornuate	49 septate, 40 bicornuate	Septate	Septate	Septate	Septate	Septate, bicornuate	Septate, subseptate
Method of surgery	Abdominal	Tompkins, Jones, Te Linde, Strassman	Tompkins, Jones, Strassman	Resectoscope	Scissors	Resectoscope	Resectoscope		
Indication	Recurrent SAB	102 recurrent SAB 42 infertility	Recurrent SAB and preterm delivery	Recurrent SAB	Recurrent SAB or preterm delivery	28 pregnancy loss or complication of pregnancy 10 infertility	2 or more SAB	2 or more SAB	First pregnancy
Live birth rate per pregnancy	39/46(84.8%)	45/66 (68%) septate 50/66 (76%) bicornuate	30/46 (65%) septate 45/54 (83%) bicornuate	63/72 (80%) 72 successful resection	60/75 (80%)	17/22 (77.3%)	33/48 (68.8%) Cumulative 51/71 (71.8%)		
Live birth rate per patient	54.9%	65.9%		61.2%		64.3%	35.1% Cumulative 54.3%	25/42 (59.5%) Cumulative 32/41 (78.0%)	8/24 (33.3%)

SAB, spontaneous abortion.

patients with a bicornuate uterus who underwent abdominal metroplasty could have live baby births, although 102 cases with recurrent spontaneous abortion (RSA) and 42 with primary infertility were included in this series. Ayhan *et al.* [25] reported that 65% (30/46) of pregnancies in women with a septate uterus and 83% (45/54) of pregnancies in women with a bicornuate uterus among the 89 cases with RSA or preterm delivery ended successfully in live births.

Hysteroscopic surgery is accepted worldwide, because of its advantages over other conventional abdominal procedures [26–30]. Goldenberg *et al.* [30] described that the pregnancy wastage in women with RSA who underwent hysteroscopic septum resection decreased from 87.5 to 44.4% postoperatively. Lee and Hickok [28] described an uncomplicated delivery rate of 77.3% after hysteroscopic septum resection in women with a preoperative pregnancy loss rate of 77.4% and miscarriage rate of 18.2%.

Kormanyos *et al.* [29] concluded that the live birth rate in cases with no remnant septum was significantly higher than that in the cases with a remnant septum after removal of a septum in patients with a history of two or more miscarriages. However, the live birth rate in patients undergoing the first hysteroscopy was 35.1% (33/94) and the cumulative live birth rate after one or two removals was 54.3% (51/94) per patient.

These studies are biased due to the fact that either there were no controls, or patients with recurrent miscarriage treated by surgery served as their own controls. Many studies have compared the live birth rates before and after surgery [16,28,30], however, it is inappropriate to simply make comparisons before and after surgery, because although the prior miscarriage rate may be 100%, the subsequent live birth rate is never 0% in cases of primary recurrent miscarriage. The infertility rate in the patients undergoing surgery is also important. The live birth rate per patient can be calculated as being between 35.1 and 65.9% (Table 1).

There have been no randomized controlled trials comparing the pregnancy outcomes between cases treated and not treated by surgery among patients with a history of recurrent miscarriage [31].

SUBSEQUENT PREGNANCY OUTCOMES IN PATIENTS WITH RECURRENT PREGNANCY LOSS CAUSED BY CONGENITAL UTERINE ANOMALIES IN THE ABSENCE OF SURGERY

Information concerning the prognosis of patients with congenital uterine anomalies not treated by surgery is limited. We conducted a case–control study of 1676 patients with a history of two or more (2–12) consecutive miscarriages whose subsequent pregnancies were ascertained at least one time in our medical records [4]. Uterine anomalies were diagnosed by HSG and laparoscopy/laparotomy.

Of the total, 54 (3.2%) had major uterine anomalies, including 38 with a partial bicornis unicolli, 10 with a septum, five with a unicornis, and one with a didelphys. Of the 42 patients (59.5%) with a septate or bicornuate uterus not treated by any kind of surgery, 25 had a successful outcome, whereas this was the case in 1096 of the 1528 (71.7%) women with normal uteri at the subsequent first pregnancy ($P=0.084$). The normal chromosomal karyotype rates in the aborted concepti in cases with anomalies (84.6%) was significantly higher than that in those without anomalies (42.5%, $P=0.006$).

Thirty-two of the remaining 41 (78.0%) patients with anomalies, because one of the 42 patients was treated by surgery after further miscarriage, and 1307 of the 1528 (85.5%) patients with normal uteri, could cumulatively have live babies within the follow-up period (not significant). Live birth rates in patients with congenital uterine anomalies tended to be lower both at the first pregnancy after diagnosis, and from the cumulative standpoint.

Ghi *et al.* [32▪] reported that 33.3% (8/24) of patients with a septate uterus gave live births at the first pregnancy. It is suggested that the dominant prevalence of complete septate uterus was the reason why the prognosis was poor.

Jayaprakasan *et al.* [33] reported pregnancy outcome in 440 individuals who underwent assisted reproductive treatment (ART). The pregnancy rates in women with arcuate uteri [36/66 (54.5%)] and major uterine anomalies [7/10 (70.0%)] were statistically similar ($P=0.09$ and $P=0.11$, respectively) to that of the matched controls with normal uteri [158/364 (43.4%)]. Although first-trimester miscarriage rates were similar ($P=0.81$) between the control group [20/158 (12.7%)] and women with arcuate uteri [5/36 (13.9%)], women with major uterine anomalies experienced a higher miscarriage rate [3/7 (42.9%); $P=0.05$]. These anomalies were not associated with a reduction in pregnancy rates following ART. However, although the arcuate uterus was not associated with an increase in first-trimester miscarriage, major uterine anomalies seemed to increase the risk of first-trimester miscarriage.

The most widely accepted theory is that the septum consists of fibroelastic tissue with inadequate vascularization, and the altered relations between myometrial and endometrial vessels exert a

negative effect on fetal placentation [34,35]. Fedele *et al.* [34] suggest on the basis of an ultrasound study that spontaneous abortion is related to the site of septal implantation.

The defect/length of the remaining uterine cavity (D/C) ratio in the miscarriage group was found to be significantly higher than that in the live birth group ($P = 0.0051$) in our study (Fig. 1) [4]. The area under the receiver operating characteristic curve, that is, the overall total diagnostic accuracy of the D/C ratio for live births, was 0.808. The results of logistic regression analysis identified a high D/C ratio as an independent risk factor for failure of live birth, after adjustment for age and the number of previous miscarriages. The odds ratio for a 0.1 increment of the D/C ratio was 1.42 (95% CI, 1.06–1.91).

Gergolet *et al.* [36▪] indicated no relevance of the height of the fundal indentation in cases of subseptate or arcuate uteri. They stated that the miscarriage rate (14.0 versus 11.1%) in both groups improved significantly after metroplasty. It must be stated again here that it is meaningless to compare the live birth rates determined before and after surgery.

CONCLUSION

There are currently no good studies that support surgery as increasing the live birth rate in cases of Müllerian anomalies. However, the prognosis might depend on the severity of the anomalies. International consensus for distinction between bicornuate and septate uteri needs to be established urgently. Good quality randomized trials with carefully classified patients are urgently needed.

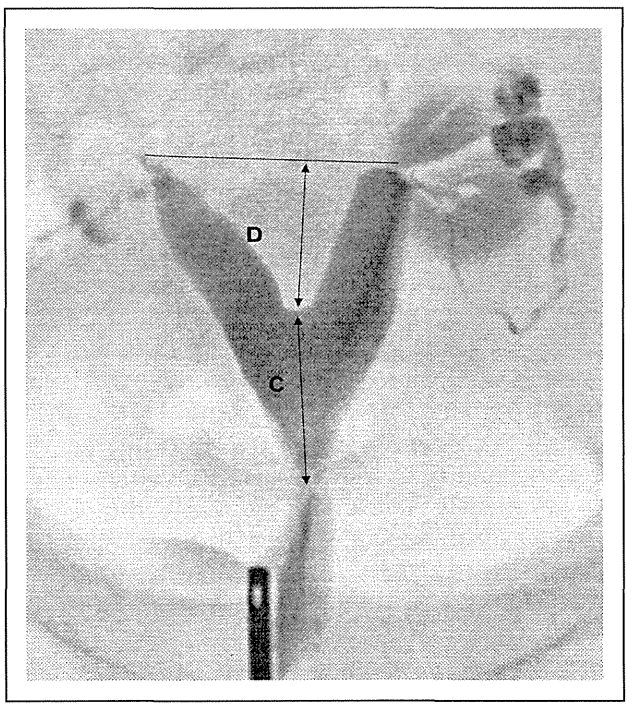

FIGURE 1. The height of the defect/length of the remaining uterine cavity ratio (D/C).

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 342).

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