from the beginning to the end of pregnancy. Yet, neither the range of normal nor the definitions of abnormal FHR are GA-specific, emphasizing the shortcomings of a single definition of fetal bradycardia (eg, FHR ≤110 bpm). Sinus bradycardia occurs in LQTS, but the molecular basis for this common occurrence is incompletely understood. Furthermore, the GA at which the sinus beat becomes bradycardic, and indeed the sensitivity and specificity of a GA-independent definition of bradycardia, are poorly understood.

Previous publications have described a range of sinus FHRs ranging from <100 to 130 bpm in LQTS fetuses.[7,3,4] As in our series, many of these LQTS fetuses with FHRs in the normal range (> 110 bpm) had a family history of LQTS. The higher FHR in those with a family history may be ascertainment bias as these subjects, screened preemptively, may be less severely affected. After birth, the majority of subjects, even those with FHR ≤110 bpm, had heart rates in the normal range.[7] Similarly, in a large study evaluating LQTS and SIDS, bradycardia in the neonate was not considered a risk factor for LQTS.[35] Thus, use of a stringent fetal bradycardia definition (ie, FHR ≤110 bpm) may result in failure to recognize fetal LQTS, and continuation of mild bradycardia (ie, heart rate >110 bpm) after birth may fail to raise the suspicion of LQTS in the neonate. This may explain why the older siblings of some fetal probands in this study with mild bradycardia were not suspected as fetuses or neonates to have LQTS.

In the absence of a known family history, the ascertainment of fetal LQTS is based on the correct and timely diagnosis of the signature LQTS rhythms. Although TdP and 2° AV block are usually easily recognized and have high specificity, they occur infrequently in the fetus with LQTS. For example, only 24% of our study cohort had these complex arrhythmias, and none of the 25 subjects in this report with LQT1 mutations had TdP or 2° AV block. Thus, it is important to identify other markers of LQTS; findings in our study suggest that FHR may be useful for this purpose. Our results show that a one size fits all FHR indicator of bradycardia will not be adequate. For example, the bradycardia index of LQT 1 subjects for FHR ≤110 bpm was only 2%, but definition of bradycardia as FHR ≤3rd percentile yielded a bradycardia index of 68%. Using a GA-independent definition of bradycardia would not have led to suspicion of LQTS in many such subjects.

Although our study group is relatively small, we found associations between FHR, rhythm phenotype, and genotype, which could be helpful in the diagnosis of LQTS. For example, individuals with *KCNQ1* mutations tended to have a mild phenotype in utero with sinus rhythm and mild bradycardia. On the other hand, genetically elusive subjects, with no known mutations and a negative family history of LQTS, had profound fetal bradycardia and complex rhythms.

Based on the results of this study, we believe that FHR ≤3rd percentile for GA is a superior definition of fetal bradycardia compared with the widely used obstetric definition. Our study suggests that the fetus with repeated FHR measurements ≤3rd percentile for GA without any other rhythm abnormality should be suspected of having LQTS. This suspicion should lead to detailed family history for LQTS. Regardless of family history, a postnatal 12-lead ECG should be examined for findings of LQTS. If the family history is positive, or the fetal proband manifests complex LQTS rhythms, ECG screening of first-degree relatives is recommended. Even if family members are asymptomatic, ECG evidence of LQTS warrants genetic testing. Finally, if postnatal genetic testing of the fetus with suspected LQTS is positive, but clinical or genetic manifestations of LQTS are negative in first-degree relatives, the possibility of parental mosaicism should be considered, especially if future pregnancies are contemplated.[36]

Table 4. Complex Fetal Rhythms in Relation to Bradycardia Index in LQTS Cohort

ID	LQTS Mutation	Fetal Rhythm	% FHR Readings ≤3rd Percentile GA	% FHR≤110 bpm
Group 1: Referral for Family History of LQTS				
2	Untested	2° AVB	100	100%
17	KCNQ1-G168R	2° AVB	100	0%
Group 2: Referral for Fetal Arrhythmia				
27	KCNH2-G628S	TdP and 2° AVB	100	84%
28	SCN5A-R1623Q	TdP and 2° AVB	100	75%
30	Uncharacterized	2° AVB	100	92%
33	KCNQ1-G314D	2° AVB	86	0%
36	SCN5A-R1623Q	TdP and 2° AVB	71	14%
40	SCN5A-L409P	TdP	100	0%
41	SCN5A-R1623Q	TdP and 2° AVB	22	0%
42	SCN5A-R1623Q	TdP and 2° AVB	11	0%

LQTS indicates long QT syndrome; TdP, torsade de pointes; AVB, atrioventricular block; GA, gestational age; and FHR, fetal heart rate.

Acknowledgments

We thank Jeannette Barczak, RN for data collection. We also acknowledge the following maternal-fetal medicine physicians for patient referral: Jude Duval, MD, Farhan Hanif, MD, Steven Ambrose, MD, and Jim Keller, MD.

Sources of Funding

This investigation was supported by the University of Utah Study Design and Biostatistics Center, with funding in part from the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through grant 8UL1TR000105 (formerly UL1RR025764).

Disclosures

None.

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CLINICAL PERSPECTIVE

Long QT syndrome (LQTS) may be as common as 1/2500 individuals, yet fewer than 100 cases have been recognized during fetal life. Fetal torsades de pointes and 2° AV block are easily attributed to LQTS. However, these complex arrhythmias are present in only 25% of fetal LQTS; the majority of LQTS fetuses have asymptomatic bradycardia that may not be recognized as an LQTS marker due to its subtle features. The standard obstetrical definition of bradycardia is fetal heart rate (FHR) $\leq$ 110 bpm. To improve recognition of perinatal LQTS we evaluated the FHR/gestational age (GA) relationship of fetal LQTS mutations versus a normal control group. We found GA dependent FHR predictors of LQTS; for example, when compared to a FHR of 110 bpm at any GA, a FHR $\leq 3^{rd}$ percentile for GA improves ascertainment of LQTS subjects from 15 to 85%. Fetuses with the lowest FHR tended to have de novo and genetically elusive LQTS mutations, and in addition to bradycardia, also manifested complex LQTS rhythms. Identification of LQTS in the fetus with a heart rate of $<3^{rd}$ also led to diagnosis of LQTS in unsuspecting family members. Thus, postnatal evaluation of individuals with a FHR $\leq 3^{rd}$percentile for GA improves ascertainment of LQTS both before and after birth.

Original Paper: Techniques

Fetal Diagnosis and Therapy

Fetal Diagn Ther 2012;32:109–115
DOI: 10.1159/000331399

Received: January 21, 2011
Accepted after revision: August 2, 2011
Published online: May 22, 2012

Comparison of PR Intervals Determined by Fetal Magnetocardiography and Pulsed Doppler Echocardiography

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Key Words
Fetal magnetocardiography · PR interval · Fetal echocardiography · Pulsed Doppler echocardiography

Abstract
Objective: In clinical practice, measurement of mechanical PR interval (mPR) with pulsed Doppler echocardiography is a standard method used to estimate the atrioventricular conduction time in the fetus. However, fetal echocardiography does not directly reflect the electrical properties of the heart. Technological advances in fetal magnetocardiography (fMCG) have allowed recording of the electrical PR interval (ePR) with high time resolution. The aim of this study was to clarify the differences between ePR and mPR. ***Methods:*** The study subjects were 295 normal human fetuses (gestational age, range 20.4–41.4 weeks) who underwent fMCG, and 135 of them underwent fetal echocardiography 15–90 min before or after fMCG. The ePR was measured using the fMCG, and the mPR was determined by two pulsed Doppler methods, simultaneous recording of the left ventricular inward and outward flow (LV in/out) (n = 135) and superior vena cava and ascending aorta (SVC/aAo) (n = 84). ***Results:*** The ePR showed a significant, but weak, positive correlation with gestational age ($r = 0.162$, $p = 0.0053$). The mPR was significantly longer than the ePR ($p < 0.0001$), with mean differences of 14.6% (95% limits of agreement −10.7, 39.9) for the LV in/out method and 14.7% (95% limits of agreement −8.6, 38.0) for the SVC/aAo method. ***Conclusion:*** Our results point to the risk of overestimation of the atrioventricular conduction time when the mPR is used, and the need for careful interpretation of PR prolongation determined by mPR.

Introduction

Assessment of the atrioventricular conduction time is important in the analysis of fetal arrhythmia. After birth, the PR interval on the surface electrocardiogram is used as a simple parameter of the atrioventricular conduction time. Technological advances in fetal magnetocardiography (fMCG) have enabled acquisition of fetal PQRST waveforms, and standardized values of the fetal electrical RR interval (eRR) and electrical PR interval (ePR) have been proposed [1–5]. However, the fMCG systems were designed for research-based use with limited clinical application. In 2003, the Japanese Ministry for Health, La-

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bor, and Welfare approved the clinical use of the Hitachi model 64-channel magnetometer (Hitachi High-Technologies Corporation, Tokyo, Japan) for fMCG. In 2008, clinical application of fMCG under the health insurance scheme started at Tsukuba University Hospital.

Fetal ultrasound cardiography (fUCG) is the most widely used modality to estimate the fetal atrioventricular conduction time [6, 7]. Measurement of the interval between the onset of blood flow induced by atrial contraction (A wave) and that of ventricular contraction (V wave) by pulsed Doppler allows estimation of the mechanical PR interval (mPR). So far, several pulsed Doppler methods have been used to measure the mPR, including simultaneous sampling of inward and outward flow of the left ventricle (LV in/out) [8, 9], reverse flow in the superior vena cava and forward flow in the ascending aorta (SVC/aAo) [10], and reverse flow in the pulmonary vein and forward flow in the pulmonary artery [11]. Pulsed Doppler-derived mPR seems to be a good surrogate for ePR. However, two reports [12, 13] that focused on the relationship between mPR and ePR measured on the signal-averaged fetal electrocardiogram demonstrated overestimation of the mPR. In the assessment of mPR in the fetus, consideration of the difference between mechanical and electrical properties is important.

The aim of this study was to clarify the difference between ePR determined by fMCG and mPR by two pulsed Doppler methods.

Material and Methods

Subjects

Between April 2008 and September 2010, 295 healthy normal human fetuses underwent fMCG. Of those, 135 fetuses underwent fUCG 15–90 min before or after fMCG. Doppler recording was performed in 135 fetuses with the LV in/out method, and in 84 fetuses the SVC/aAo method was also used. Fetuses with structural heart disease, arrhythmia, or maternal disease (e.g. diabetes mellitus, autoimmune diseases) were excluded from the study.

Instrumentation and Measurements

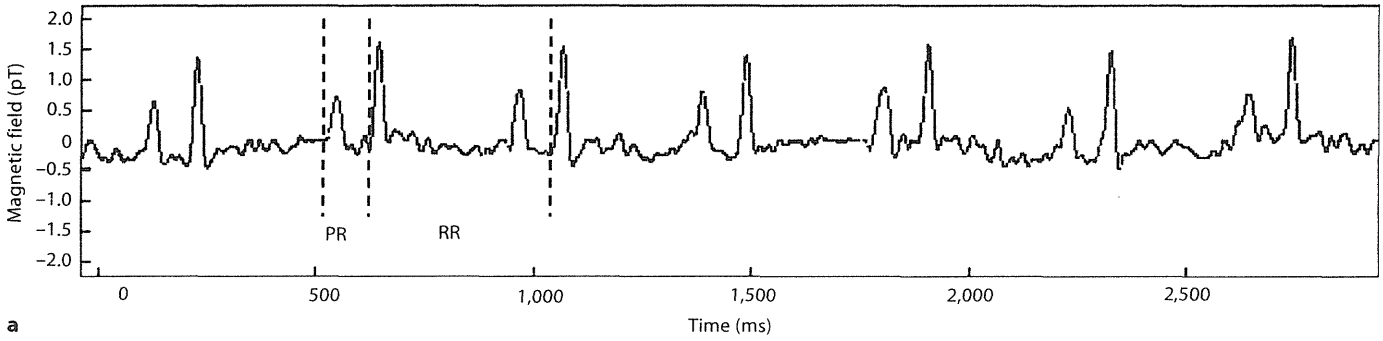
A 64-channel superconducting quantum interference device (SQUID) system (MC-6400, Hitachi High-Technologies Corporation) housed in a magnetically shielded room was used in this study. This system was designed for use in both adults and fetuses. The SQUID sensors were distributed at 8 × 8 points with an interval of 25 mm, covering an area of 175 × 175 mm. The co-axial 64 sensors detect the tangential component of magnetic fields generated by the fetal heart. Magnetic signals were acquired at a sampling rate of 1,000 Hz for 120–240 s, and the signals were passed through a band-pass filter (0.1–100 Hz) and a power-line noise filter (50 Hz). The fMCG was recorded by positioning the maternal abdominal surface to the sensor as close as possible. Immediately before recording, the fetal heart position and the distance between the maternal abdominal surface and the anterior surface of the fetal ventricle were determined by a portable echocardiographic machine. The fMCG was recorded 2–4 times in one session, with different maternal positions if necessary. The ePR and eRR were measured at the baseline HR beats, and the averaged values of more than 3 consecutive beats were calculated in this study (fig 1a). The onset and offset of the P wave, QRS complex, and T wave were determined manually using MCG analysis software (Hitachi High-Technologies Corporation). Recording and measurement of fMCG was performed by a single observer (Y.K.) without knowledge of the fUCG findings. Two-dimensional and pulsed Doppler fUCG were performed in each case using the SONOS-5500 (Philips, Andover, Mass., USA) for the screening of structural heart disease and measurement of parameters, and the acquired pulsed Doppler waveforms were stored in the optical disk recording system. The mPR and mechanical RR interval (mRR) were determined by the LV in/out methods and the SVC/aAo method was also used if time permitted. LV in/out was recorded by placing the Doppler sample volume at the junction of the anterior leaflet of the mitral valve and left ventricular outflow tract in an apical 5-chamber view, by simultaneous recording of the inflow and outflow of the left ventricles (fig. 1b) [8, 9]. The SVC/aAo was recorded by placing the Doppler sample at the point where the SVC and aAo adjoined each other (fig. 1c) [10, 14, 15]. Recording and measurement of fUCG was performed by another single observer (M.T.-I.) who did not know the fMCG findings. Due to limitations of time in our outpatient clinic, all of the fUCG examinations, including screening for structural heart diseases, acquisition of the Doppler waveforms, and measurement parameters, were performed within 20 min.

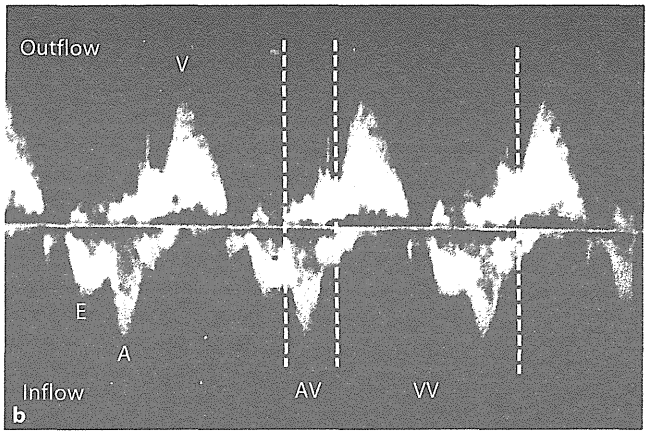
Interobserver and Intraobserver Variations

For assessment of interobserver variation, certain parameters were measured again in 30 randomly selected fetuses by another observer (T.I.) for fMCG and (Y.K.) for fUCG. For assessment of intraobserver variation, the first observer remeasured the ePR and mPR in 30 randomly selected fetuses with a time interval of more than 3 months.

Statistical Analysis

Continuous values are expressed as means ± SD. Student's unpaired t test was used to assess differences in gestational age between the fetuses in whom mPR could be determined and those in whom it could not. The correlations between ePR and gestational age, as well as eRR and gestational age, were computed using linear regression analysis. Stepwise multiple regression analysis was used to assess the effect of eRR, estimated fetal body weight, and gestational age on ePR. Direct comparisons between ePR and mPR were made using Student's paired t test. Bland-Altman analysis was used to display the bias and the limits of agreement between ePR and mPR [16]. Stepwise multiple regression analysis was used to assess the effect of gestational age, eRR, and differences between eRR and mRR on the differences between ePR and mPR. Bland-Altman analysis was also used for assessment of interobserver and intraobserver variations. $p < 5\%$ and $F > 2.0$ was considered statistically significant. All statistical analyses were performed using StatView 5.0 software (SAS Institute, Inc., Cary, N.C., USA).





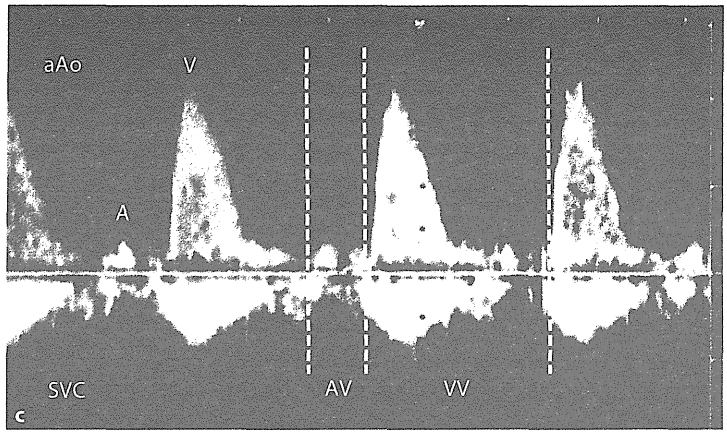


Fig. 1. Measurement of PR and RR intervals. **a** Waveforms of raw data of fMCG. The ePR represents the interval between the onsets of the P wave and the QRS complex, while the RR interval represents the interval between the onsets of two consecutive QRS complexes. **b** Waveforms of simultaneous recording of inward and outward flow of the left ventricle by the pulsed Doppler method. The mPR represents the interval between the onsets of the A wave of inflow and the V wave of outflow. **c** Waveforms of simultaneous recording of bidirectional flow of the SVC and forward flow of the aAo by the pulsed Doppler method. The mPR represents the interval between the onsets of the reversal A wave in SVC and the V wave of aAo.

Results

The success rates of determination of PR intervals were 100% with fMCG (n = 295), 88.9% with the LV in/out method (n = 135), and 94.0% with the SVC/aAo method (n = 84). The mean ePR was 100.2 ± 15.7 ms (range 64–143). The mean mPR was 119.6 ± 12.3 ms (range 85–150) in the LV in/out method and 120.2 ± 12.1 ms (range 93–150) in the SVC/aAo method. The success rate of determination of the PR interval and the distribution of the gestational age of fetuses are summarized in table 1. There were no significant differences in gestational age between the fetuses in whom mPR could be determined and those in whom it could not for both the LV in/out method (p = 0.71) and the SVC/aAo method (p = 0.85). A significant, but weak, positive correlation was noted between ePR and gestational age (r = 0.162, p = 0.0053), and between eRR and gestational age (r = 0.232, p < 0.0001) (fig. 2a, b). Stepwise multiple regression analysis indicated that only gestational age significantly but weakly affected ePR (r = 0.159, F = 6.134).

Table 1. Distribution of the gestational age of fetuses and success rate of determination of PR intervals

Gestational age	ePR (%)	mPR (%)	
		LV in/out	SVC/aAo
≤28 weeks	71/71 (100)	39/41 (95.1)	24/26 (92.3)
29–35 weeks	155/155 (100)	70/80 (86.3)	43/45 (95.6)
36–42 weeks	69/69 (100)	12/14 (85.7)	12/13 (92.3)
Overall	295/295 (100)	120/135 (88.9)	79/84 (94.0)

The results of paired comparisons of ePR with mPR, and eRR with mRR, are summarized in tables 2 and 3.

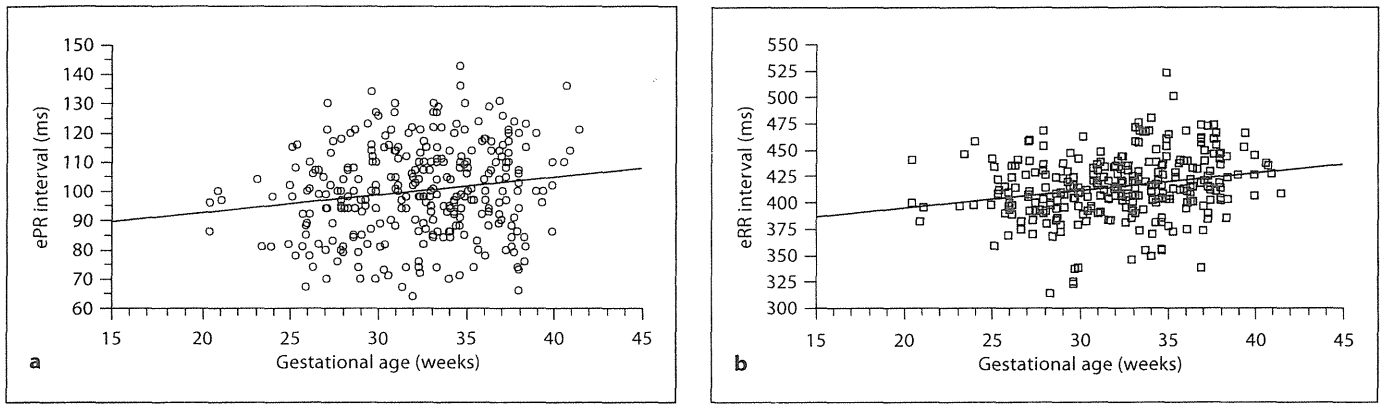


Fig. 2. Relationship between gestational age and the ePR interval (**a**) and eRR interval (**b**) by fMCG. The ePR and eRR intervals correlated significantly but weakly with gestational age (ePR: n = 295, y = 80.7 ± 0.60x, r = 0.162, p = 0.0053; eRR: n = 295, y = 362.6 ± 1.65x, r = 0.232, p < 0.0001).

Table 2. Comparisons of RR and PR intervals measured by fMCG and fUCG (LV in/out) (n = 120)

	fMCG	fUCG	Paired t test
RR interval, ms	414.8 ± 24.3	419.0 ± 27.6	p = 0.120
PR interval, ms	101.7 ± 15.8	119.6 ± 12.4	p < 0.0001

Table 3. Comparisons of RR and PR intervals measured by fMCG and fUCG (SVC/aAo) (n = 79)

	fMCG	fUCG	Paired t test
RR interval, ms	411.0 ± 5.0	414.1 ± 29.5	p = 0.381
PR interval, ms	102.0 ± 14.1	120.2 ± 12.1	p < 0.0001

Student's paired t test revealed significant differences between the ePR and mPR determined by LV in/out ($p < 0.0001$) and SVC/aAo ($p < 0.0001$). Bland-Altman analysis for comparison of mPR and ePR showed a mean difference of 14.6% (95% limits of agreement –10.7, 39.9) for LV in/out and 14.7% (95% limits of agreement –8.6, 38.0) for SVC/aAo (fig. 3a, b). On the other hand, there were no significant differences between mRR and eRR (fig. 3c, d). Stepwise multiple regression analysis indicated that the difference between the ePR and the mPR was not significantly affected by any factor.

The mean interobserver difference in PR interval was 1.0% (95% limits of agreement –6.0, 8.1) for fMCG, 0.4% (95% limits of agreement –10.6, 11.4) for LV in/out, and 2.3% (95% limits of agreement –10.6, 15.1) for SVC/aAo. The mean intraobserver difference in PR interval was –0.1% (95% limits of agreement –6.4, 6.2) for fMCG, 1.0% (95% limits of agreement –8.9, 10.9) for LV in/out, and 2.2% (95% limits of agreement –8.0, 12.3) for SVC/aAo.

Discussion

The PR interval encompasses the conduction time of the atrium (sinus node to entrance of atrioventricular node), the atrioventricular node, and the His-Purkinje system. These conduction properties depend on various factors such as heart size, the extent of autonomic nervous system development, and myocardial damage. Our results demonstrated that ePR showed a significant, but weak, correlation with gestational age. Previous studies described prolongation of ePR measured by fMCG [2–4] and mPR measured by fUCG [17, 18] with increase in gestational age. This phenomenon seems to be reasonable because the fetal heart size becomes larger and parasympathetic function develops with increasing gestational age, as is the case with normal postnatal development. However, some controversy exists on this point. Some reports showed that PR intervals are independent of gestational age [9, 19, 20].

Our results demonstrated overestimation of pulsed Doppler-derived mPR compared with ePR determined by fMCG. This finding was similar to those of previous

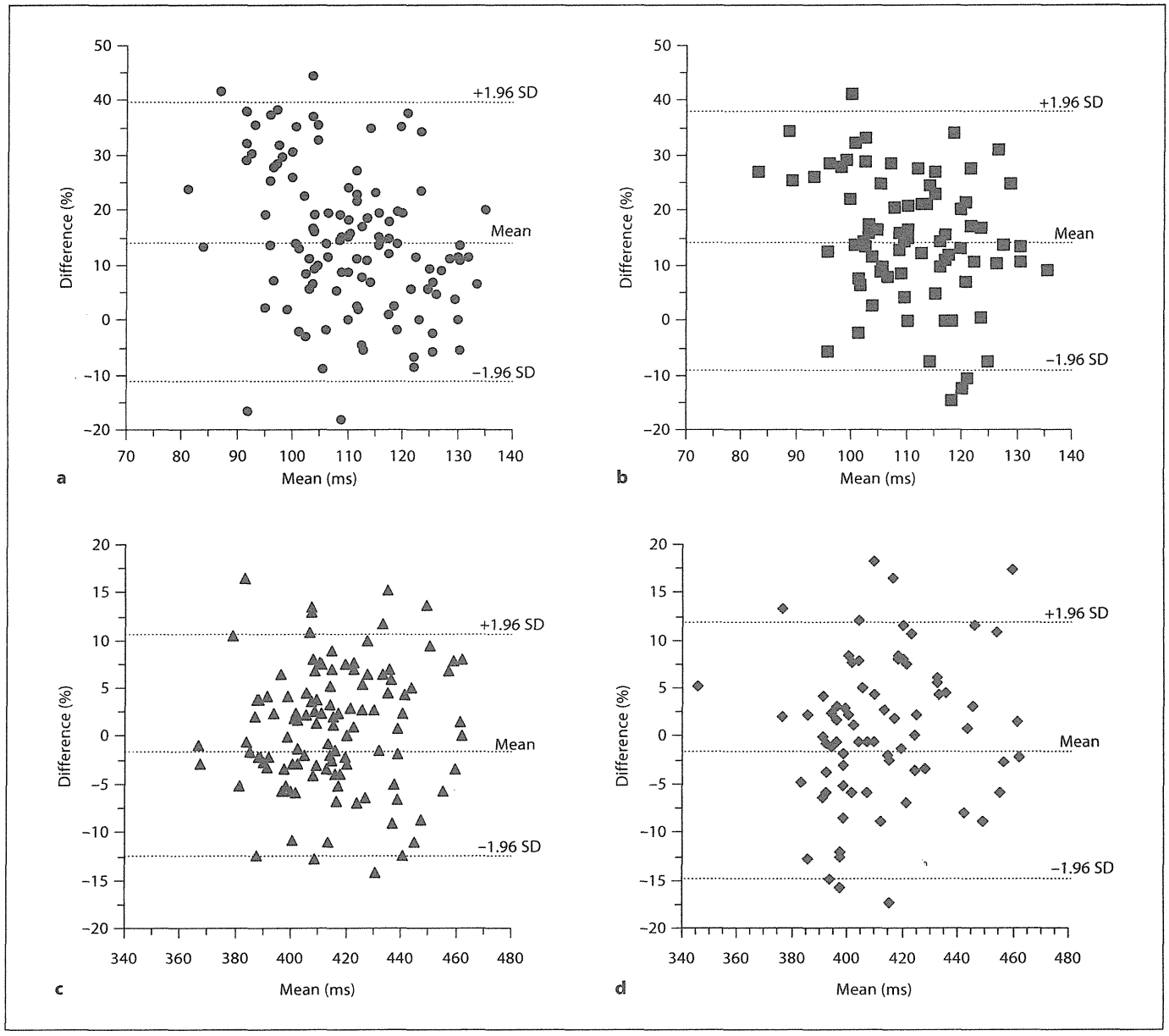


Fig. 3. Results of the Bland-Altman plot analysis for agreement between PR and RR intervals determined by fUCG and fMCG. **a** mPR measured by LV in/out versus ePR measured by fMCG. **b** mPR measured by SVC/aAo versus ePR. **c** mRR measured by LV in/out versus eRR by fMCG. **d** mRR measured by SVC/aAo versus eRR. The mean differences between mPR and ePR were 14.6% (95% limits of agreement −10.7, 39.9) for the LV in/out method and 14.7% (95% limits of agreement −8.6, 38.0) for the SVC/aAo method, and they were significantly different from 0. On the other hand, the mean differences between mRR and eRR were not significantly different from 0. The mean differences between mRR and eRR were 0.6% (95% limits of agreement −0.6, 1.8) for the LV in/out method and 0.4% (95% limits of agreement −0.4, 2.2) for the SVC/aAo method.

studies performed with fetal electrocardiography [12, 13]. Such a difference in the estimated values is not unreasonable because fUCG directly reflects the mechanical properties of the heart, rather than its electrical properties. The sum of the electromechanical delay (the interval between electrical myocardial excitation and myocardial contraction) and the isovolumic contraction time (the time between the onset of myocardial contraction and blood flow) should explain the time difference between the onsets of the P wave and A wave, as well as the QRS

complex and V wave [21]. This electro-hemodynamic delay, the preejection period, is influenced by various hemodynamic factors, such as heart rate, preload and afterload conditions, and myocardial contractility. Comparison of the simultaneously recorded ePR and mPR in neonates indicated that the preejection period of the ventricle was longer than that of the atrium [21]. Based on the data of neonates, this difference in the preejection period of the atrium and ventricle may be the main factor for the time difference between ePR and mPR. Therefore, the prolonged mPR might be due not only to delays in atrioventricular conduction but also to changes in the hemodynamic condition. Several studies have reported the advantages of tissue Doppler imaging compared with pulsed Doppler assessment: the former is less influenced by the isovolumic contraction time in assessment of the atrioventricular conduction time [12, 22]. Although there is no established standardized method for fetal assessment, especially with regard to the location of the sampling points for the ventricle [12, 22], tissue Doppler imaging may be a potentially suitable alternative method for estimation of ePR.

In our study, the absolute difference between ePR and mPR ranged from -18.0 to 59.0 ms in LV in/out and from -16.0 to 52.0 ms in SVC/aAo; these values are similar to those of other studies using signal-averaged fetal electrocardiography [12, 13]. These wide variations could not be fully explained by differences in electrical and mechanical properties. Although there was no significant difference between the eRR and mRR values, ePR and mPR were measured on different heartbeats, and it is possible that these intervals fluctuated beat by beat. Mensah-Brown et al. [23] succeeded in simultaneous recording of fMCG and fUCG to calculate the fetal ventricular preejection time. However, this method is not easy to apply for determination of the atrial preejection time. The combination of fetal electrical and hemodynamic data on the same heart beats should provide a detailed description of the electro-hemodynamic relations, such as the preejection period, and should clarify the mechanism underlying the difference between ePR and mPR.

Standard values for ePR and mPR have been proposed for each gestational period [2–4, 17, 18]. However, the diagnostic criteria for the first-degree atrioventricular block in the fetus are still not clear. Such criteria are important in the fetuses of anti-SSA/SSB antibody-positive mothers because early detection of congenital atrioventricular block would be useful for prevention of its progression to a higher grade [24, 25]. We believe that fMCG is a powerful tool for the detection of atrioventricular block with less effect of hemodynamic factors; however, fMCG is available only in a limited number of hospitals, and fUCG is still the most practical modality to detect atrioventricular block in the fetus. When the mPR becomes longer during the course of pregnancy, investigators should examine the presence of not only damage of the conduction system but also changes in hemodynamics.

Limitation of the Study

Although there was no relationship between gestational age and the success rate of determination of pulsed Doppler-derived mPR in the present study, the number of subjects was not enough due to the retrospective nature of the study. As the LV in/out method was relatively independent of fetal position and not time-consuming compared with the SVC/aAo method, we first applied the former method and then attempted the latter method if time permitted. To compare the values of mPR obtained by both methods, a prospective study in a large number of subjects is needed.

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Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Pediatric Cardiology and Adult Congenital Heart Disease

Evaluation of Transplacental Treatment for Fetal Congenital Bradyarrhythmia

– Nationwide Survey in Japan –

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Background: There are few large studies of fetal congenital bradyarrhythmia. The aim of the present study was to investigate the effects and risks of transplacental treatment for this condition.

Methods and Results: Using questionnaires, 128 cases of fetal bradyarrhythmia were identified at 52 Japanese institutions from 2002 to 2008. Of the 128 fetuses, 90 had structurally normal hearts. Among these 90 fetuses, 61 had complete atrioventricular block (CAVB), 16 had second-degree AVB, 8 had sinus bradycardia, and 5 had other conditions. The 61 CAVB fetuses were divided into those who did (n=38) and those who did not (n=23) receive transplacental medication. Monotherapy with β-sympathomimetics, steroid monotherapy, and combination therapy with these agents was given in 11, 5 and 22 cases, respectively. Beta-sympathomimetics improved bradycardia (P<0.001), but no medication could significantly improve the survival rate. Fetal hydrops was associated with a 14-fold increased risk of perinatal death (P=0.001), and myocardial dysfunction was a significant risk factor for poor prognosis (P=0.034). Many adverse effects were observed with steroid treatment, with fetal growth restriction increasing significantly after >10 weeks on steroids (P=0.043).

Conclusions: Treatment with β-sympathomimetics improved bradycardia, but survival rate did not differ significantly in fetuses with and without transplacental medication. It is recommended that steroid use should be limited to <10 weeks to avoid maternal and fetal adverse effects, especially fetal growth restriction and oligohydramnios. (*Circ J* 2012; **76**: 469–476)

Key Words: Anti-Ro/SSA antibody; Congenital atrioventricular block; Pregnancy; Steroids; Transplacental treatment

Fetal congenital bradyarrhythmia is an uncommon but life-threatening disease, especially in the case of complete atrioventricular block (CAVB), which has a poor prognosis because of fetal hydrops, endocardial fibroelastosis and late-onset dilated cardiomyopathy.[1–9] Predominantly untreated CAVB has a significant mortality rate of 14–34%, while congenital CAVB is irreversible and requires a pacemaker in approximately 66% of cases after birth.[10] The association of CAVB with maternal anti-Ro/Sjögren's syndrome A (SSA) antibodies is well established, but the trigger for the maternal antibody interaction with the fetal Ro particle is unknown in some cases of antibody-exposed babies.[2,7–9,11,12]

There is limited evidence for the clinical efficacy of transplacental treatment of congenital AVB.[13–19] Steroids and i.v. immunoglobulins are given as anti-inflammatory treatment, while β-sympathomimetics are used for fetal pacing.[20] A recent

Received September 8, 2011; revised manuscript received October 27, 2011; accepted November 1, 2011; released online December 23, 2011 Time for primary review: 18 days

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ISSN-1346-9843 doi:10.1253/circj.CJ-11-1020

Table 1. Baseline Characteristics of CAVB Fetuses

	Medication group (n=38)	No medication group (n=23)	P value
Maternal anti-SSA antibodies	29 (76.3)	11 (47.8)	<0.05‡
Gestational age at diagnosis (weeks)	24±3.2	28±5.7	<0.005†
Fetal heart rate at diagnosis (beats/min)	58±7.9	63±14.7	NS†
Fetal hydrops	16 (42.1)	6 (26.1)	NS‡
Fetal myocardial dysfunction	13 (34.2)	7 (30.4)	NS‡
Gestational age at initiation of therapy (weeks)	26±3.6	-	
Fetal heart rate at initiation of therapy (beats/min)	56±8.4	–	
Gestational age at delivery (weeks)	34±4.0	35±4.5	NS†
Birth weight (g)	2,120±620	2,528±653	<0.001†
Delivery mode			
Vaginal	8	7	NS‡
Cesarean section	30	16	NS‡
Permanent pacemaker implantation	14 (46.7)	6 (35.3)	NS‡
Neonatal survival	30 (78.9)	17 (73.9)	NS‡

Data given as mean±SD or n (%). P<0.05, significant difference.
†Student's t-test; ‡chi-square test and Fisher's exact test.
CAVB, complete atrioventricular block; SSA, Sjögren's syndrome A.

cohort study found an improved survival rate of >90% with initiation of maternal high-dose dexamethasone at the time of CAVB detection, and maintenance of this drug during pregnancy with use of β-sympathomimetics to keep fetal heart rates at >55 beats/min.[9,21] It was also suggested that prolonged use of dexamethasone might render fetuses with congenital CAVB less likely to develop the additional manifestations of myocarditis, cardiomyopathy, and hydrops fetalis, thus improving the overall outcome. Use of steroids, however, is controversial because of the potential risks for the fetus, including problems with neurological development, growth retardation, and oligohydramnios.[22-25]

Few large studies of fetal congenital bradyarrhythmia have been performed in Japan. The aims of the present study were to determine the features of fetal congenital bradyarrhythmia in Japan, and to examine the effects and risks of transplacental treatment for this condition.

Methods

Subjects

Data were collected using questionnaires sent to Departments of Perinatology and Pediatric Cardiology at 750 institutions in Japan over 7 years (2002–2008). The response rate was 60.7% (455 institutions). Fetal bradyarrhythmia was defined as ventricular heart rate <100 beats/min at the time of diagnosis.[4] The following perinatal data were also collected: gestational age at diagnosis and delivery, presence or absence of a congenital heart defect (CHD), type of bradyarrhythmia, method of diagnosis, presence or absence of maternal autoantibodies such as anti-Ro/SSA antibodies, presence or absence of fetal hydrops, presence or absence of fetal myocardial dysfunction, fetal ventricular and atrial heart rate at presentation, prenatal treatment, mode of delivery, and outcome. Adverse effects related to prenatal treatment were also evaluated.

Statistical Analysis

Statistical analysis was performed using STATA 11.1 (StataCorp, College Station, TX, USA) and JMP 9 (SAS Institute, Cary, NC, USA). Data are presented as mean±SD or number of patients and were analyzed using Student's t-test. Categorical variables were evaluated on chi-square test and Fisher's exact test. Logistic regression was used to adjust for baseline variables known to be associated with fetal ventricular heart rate, fetal hydrops, fetal myocardial dysfunction, and maternal anti-Ro/SSA antibody. Time to fetal or neonatal death was analyzed using the Kaplan-Meier method with a log-rank test and a Cox proportional hazard model. P<0.05 was considered significant.

Results

Baseline Characteristics

A total of 128 cases were registered from 52 institutions during 7 years (2002–2008). All cases of fetal bradyarrhythmia were diagnosed during fetal life using echocardiography. In 8 cases, magnetocardiography was performed due to fetal bradyarrhythmia and family history of long QT syndrome (LQTS). Of the 128 fetuses, 38 (29.7%) had CHD, 15 had left atrial isomerism, 1 had right atrial isomerism, 5 had atrioventricular septal defect, 4 had corrected transposition of the great arteries, 4 had pulmonary stenosis, and 9 had other conditions. Patent ductus arteriosus and atrial septal defect were categorized as an absence of CHD. Ninety fetuses (70.3%) had a structurally normal heart, of whom 61 had CAVB, 16 had second-degree AVB, 8 had sinus bradycardia, 3 had sick sinus syndrome. Nine LQTS cases occurred in combination with another condition.

CAVB

Of the 61 fetuses with a structurally normal heart and CAVB (Table 1), 38 received transplacental medication. No fetus showed improvement of heart block. Monotherapy with β-sympathomimetics was given in 11 cases, steroids were given in 5 cases, and combination therapy with these agents was used in 22 cases. No transplacental medication was given in 23 cases. Ritodrine hydrochloride was used as the β-sympathomimetic agent. Steroids tended to be used in fetuses that were positive for maternal anti-Ro/SSA antibody throughout pregnancy, but the chosen steroid differed among institutions. Maternal i.v. immunoglobulin was not used. After birth, a pacemaker was implanted based on the Japanese guidelines of syncope, ventricular heart rate <50 beats/min, decreased cardiac function, LQTS, and a sudden pause longer than 2–3-fold the regular ventricular heart rate.

Table 2. Factors in Improvement of Bradycardia

	OR	95%CI	P value
β-sympathomimetics	49.02	5.18–464.02	<0.005
Steroids	1.32	0.24–7.20	0.745
β-sympathomimetics+steroids	725,448.8	0	0.996
Fetal heart rate	1	0.93–1.08	0.924
Fetal hydrops	0.41	0.07–2.39	0.319
Fetal myocardial dysfunction	1.14	0.20–6.60	0.883
Maternal anti-Ro/SSA antibodies	0.22	0.04–1.36	0.105

P<0.05, significant difference.
Logistic regression was used to adjust for baseline variables known to be associated with fetal ventricular heart rate, fetal hydrops, fetal myocardial dysfunction, and maternal anti-Ro/SSA antibody.
OR, odds ratio; CI, confidence interval; SSA, Sjögren's syndrome A.

Table 3. Factors in Fetal or Neonatal Death

	HR	95%CI	P value
β-sympathomimetics	1.16	0.37–3.63	0.792
Steroids	0.56	0.20–1.58	0.273
Fetal heart rate	0.98	0.92–1.05	0.546
Fetal hydrops	13.84	3.12–61.44	0.001
Fetal myocardial dysfunction	2.44	0.71–8.40	0.157
Maternal anti-Ro/SSA antibodies	1.07	0.33–3.47	0.906

P<0.05, significant difference.
Logistic regression was used to adjust for baseline variables known to be associated with fetal ventricular heart rate, fetal hydrops, fetal myocardial dysfunction, and maternal anti-Ro/SSA antibody.
HR, hazard ratio; CI, confidence interval; SSA, Sjögren's syndrome A.

Table 4. Factors in Development of Fetal Hydrops

	OR	95%CI	P value
β-sympathomimetics	2	0.35–11.50	0.439
Steroids	0.27	0.04–1.97	0.198
Fetal heart rate	1.01	0.94–1.08	0.813
Fetal myocardial dysfunction	5.71	1.14–28.62	0.034
Maternal anti-Ro/SSA antibodies	0.71	0.13–3.90	0.698

P<0.05, significant difference.
Logistic regression was used to adjust for baseline variables known to be associated with fetal ventricular heart rate, fetal myocardial dysfunction, and maternal anti-Ro/SSA antibody.
OR, odds ratio; CI, confidence interval; SSA, Sjögren's syndrome A.

The anti-Ro/SSA antibody-positive rate was significantly higher in fetuses treated with transplacental medication compared to those who did not receive this medication (76.3% vs. 47.8%; P=0.031). Gestational age at diagnosis was significantly lower in those receiving transplacental medication (24.0 weeks vs. 28.3 weeks; P=0.003). Fetal ventricular heart rate at diagnosis did not differ between the 2 groups, but the ventricular heart rate was significantly lower in fetuses treated with transplacental medication (56 beats/min vs. 63 beats/min; P=0.034). Birth weight was also significantly lower in fetuses treated with transplacental medication (2,120 g vs. 2,528 g; P=0.006). Gestational age at delivery, neonatal survival rate, and pacemaker implantation rate did not differ between the 2 groups.

Multivariate analysis was performed with adjustment for baseline variables with a known association with fetal ventricular heart rate, fetal hydrops, fetal myocardial dysfunction, and the presence of maternal anti-Ro/SSA antibodies (**Tables 2–4**). In this analysis, β-sympathomimetic treatment was significantly associated with improved bradycardia (odds ratio [OR], 49.02; 95% confidence interval [CI]: 5.18–464.02; P<0.001), whereas steroids were ineffective, and no evidence of a synergistic effect was obtained. The presence of maternal anti-Ro/SSA antibodies may inhibit improvement of bradycardia, but this effect was not significant (OR, 0.22; 95%CI: 0.04–1.36; P=0.105). Drug therapy had no significant effect on survival. Fetal ventricular heart rate and the presence of maternal anti-Ro/SSA antibodies also had no influence on prognosis, but fetal hydrops was associated with a 14-fold increased risk of perinatal death (hazard ratio [HR], 13.84; 95%CI: 3.12–61.44; P=0.001).

Kaplan-Meier survival curves are shown in **Figure**. The primary endpoint was intrauterine death or neonatal death. Beta-sympathomimetic treatment was not associated with improved prognosis. Steroid also did not improve the prognosis (HR, 0.56; 95%CI: 0.20–1.58; P=0.273). Fetal myocardial dysfunction was a significant risk factor for fetal hydrops (OR, 5.71; 95%CI: 1.14–28.62; P=0.034). Fetal ventricular heart rate and the presence of maternal anti-Ro/SSA antibodies were not associated with fetal hydrops. Beta-sympathomimetic treatment did not inhibit development of fetal hydrops. Steroids tended to inhibit fetal hydrops, but again this effect was not

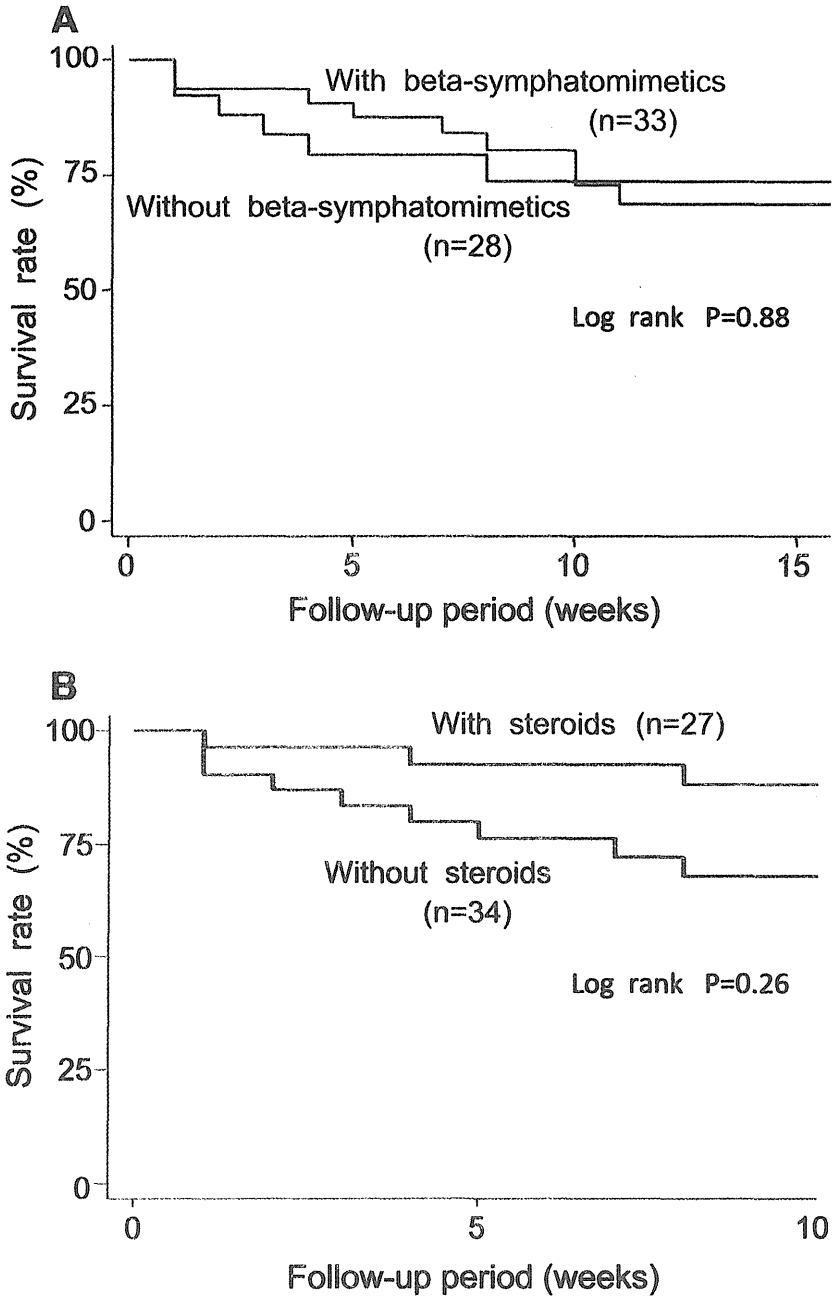

Figure. Kaplan-Meier survival curves (log-rank test and Cox proportional hazard model) for the primary endpoint (fetal death or neonatal death) in fetuses treated (**A**) with and without β-sympathomimetics and (**B**) with and without steroids. Follow-up period, time from diagnosis in utero to fetal or neonatal death. Significant difference defined as $P<0.05$.

statistically significant (OR, 0.27; 95%CI: 0.04–1.97; P=0.198). Drug therapy had no significant effect on improvement of fetal myocardial dysfunction.

Second-Degree AVB With Bradycardia

Of the 90 fetuses with a structurally normal heart, second-degree AVB was present in 16 cases (**Table 5**). Transplacental medication was given in 8 of these cases: β-sympathomimetic monotherapy in 4, steroids in 3, and a combination of these therapies in 1. In the 8 medication cases, fetal ventricular heart rate at diagnosis was significantly lower than that in the non-medication cases (70 beats/min vs. 79 beats/min; P=0.017). No other clinical characteristics differed significantly between the 2 groups. Of the 8 medicated fetuses, 3 developed CAVB, 3 maintained second-degree AVB, 1 improved to first-degree AVB, and 1 had no AVB at the time of delivery. Of the 8 non-medicated fetuses, 2 developed CAVB, 3 maintained second-degree AVB, and 3 had no AVB at the time of delivery. Survival rate did not differ between the groups (87.5%).

Adverse Effects of Transplacental Treatment

Treatment-related adverse events were examined in the 63 fetuses with a structurally normal heart and no fetal hydrops (**Table 6**). Steroids were given in 23 cases, drugs other than steroids were given in 10 cases, and no treatment was given in 30 cases. Gestational age at delivery did not differ among these 3 groups. In the steroid group, birth weight was significantly lower than in the non-treatment group (2,201 g vs.

Table 5. Second-Degree AVB Fetus Baseline Characteristics vs. Medication

	Medication (n=8)	No medication (n=8)	P value
Maternal anti-Ro/SSA antibodies	4	3	NS‡
Gestational age at diagnosis (weeks)	28±4.3	26±5.0	NS†
Fetal heart rate at diagnosis (beats/min)	70±9.0	79±10.4	<0.05†
Fetal hydrops	2	2	NS‡
Fetal myocardial dysfunction	3	2	NS‡
Gestational age at initiation of therapy (weeks)	29±4.8	–	
Fetal heart rate at initiation of therapy (beats/min)	70±10.0	–	
Gestational age at delivery (weeks)	35±3.8	37±2.1	NS†
Birth weight (g)	2,207±688	2,533±544	NS†
Delivery mode			
Vaginal	2	5	NS‡
Cesarean section	6	3	NS‡
Degree of AVB at delivery			
Complete	3	2	NS‡
Second	3	3	NS‡
First	1	0	NS‡
None	1	3	NS‡
Neonatal survival	7 (87.5)	7 (87.5)	NS‡

Data given as mean±SD or n (%).
P<0.05, significant difference. †Wilcoxon test; ‡chi-square test and Fisher's exact test.
AVB, atrioventricular block; SSA, Sjögren's syndrome A.

Table 6. Baseline Characteristics vs. Treatment Type†

	Steroid treatment (n=23)	Non-steroid treatment (n=10)	No treatment (n=30)
Treatment (weeks)	8.8±4.4	5.6±3.2	–
Gestational age at delivery (weeks)	36±2.6	35.8±2.6	36.8±3.0
Birth weight (g)	2,201±525*	2,413±552	2,713±512*
Fetal arrythmia: CAVB	21	6	23
Fetal arrythmia: Second-degree AVB	1	2	5
Maternal diabetes	1 (4.3)	0	0
Fetal growth restriction	6 (26.1)	0	2 (6.7)
Fetal oligohydramnios	2 (8.7)	0	0
Neonatal adrenal insufficiency	1 (4.3)	0	0

Data given as mean±SD or n (%).
†For fetuses without fetal hydrops and with a structurally normal heart. *P<0.05 (Student's t-test).
CAVB, complete atrioventricular block; AVB, atrioventricular block.

Table 7. Baseline Characteristics vs. Length of Steroid Treatment

	<10 weeks (n=12)	≥10 weeks (n=11)	P value
Treatment (weeks)	5.4±2.7	12.5±2.5	<0.01†
Gestational age at delivery (weeks)	35±3.2	36±1.7	NS†
Birth weight (g)	2,184±569	2,218±503	NS†
Maternal diabetes	0	1 (9.1)	NS‡
Fetal growth restriction	1 (8.3)	5 (45.5)	<0.05‡
Fetal oligohydramnios	0	2 (18.2)	NS‡
Neonatal adrenal insufficiency	0	1 (9.1)	NS‡

Data given as mean±SD or n (%). P<0.05, significant difference.
†Student's t-test; ‡chi-square test and Fisher's exact test.

2,713 g; P=0.001) and fetal growth restriction was close to being significantly higher than in the non-steroid (26.1% vs. 0%; P=0.050) and non-treatment (26.1% vs. 6.7%; P=0.074) groups. Adverse effects that might have been attributable to the use of steroids included development of oligohydramnios in 8.7% of cases, maternal diabetes in 4.3%, and neonatal adrenal insufficiency in 4.3%. All these adverse effects were observed in cases of steroid use >10 weeks (**Table 7**). In particular, fetal growth restriction increased significantly after steroid use >10 weeks (45.5% vs. 8.3%; P=0.043).

LQTS

Of the 90 fetuses with a structurally normal heart, 9 (10.0%) were diagnosed with LQTS, including 4 diagnosed on electrocardiography after birth and 5 diagnosed on magnetocardiography during fetal life. The background of the LQTS fetuses included a family history of LQTS (n=2), maternal anti-Ro/SSA antibody (n=2), fetal hydrops (n=3), myocardial dysfunction (n=2), CAVB (n=6), second-degree AVB with bradycardia (n=1), and sinus bradycardia (n=2). In 4 of the 9 cases of LQTS, emergency cesarean section was performed because of fetal ventricular tachycardia/torsades de pointes (VT/TdP) at 33–36 weeks of gestation. In 2 of the 9 cases, fetal hydrops caused neonate death.

Discussion

This is the first large-scale study to investigate the effects and risks of transplacental treatment for fetal congenital bradyarrhythmia in Japan. The results indicate that fetal hydrops is associated with a 14-fold increased risk of perinatal death, and that fetal myocardial dysfunction is a significant risk factor for fetal hydrops. Fetal ventricular heart rate and the presence of maternal anti-Ro/SSA antibodies were not associated with neonatal prognosis. Beta-sympathomimetics improved bradycardia, but survival rate did not differ significantly with regard to transplacental medication. Maternal and fetal adverse effects were observed in cases of steroid use. In particular, fetal growth restriction increased significantly after steroid use >10 weeks.

Evaluation of Anti-Ro/SSA Antibodies

Ro/SSA is one of the major immunogenic ribonucleoproteins, and antibodies against these proteins are found in a number of connective diseases, especially in Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE). Anti-Ro/SSA antibodies are detected in 60–90% of SS cases and in 30–50% of SLE cases.[26,27] Interestingly, these antibodies are relatively common and are detected in 1–2% of randomly tested pregnant women.[28] Currently, the outcome of anti-Ro/SSA-positive pregnancies is very good when prospectively followed by multidisciplinary teams with experience in this field.[1] Transplacental passage of anti-Ro/SSA antibodies from mother to fetus, however, is associated with a risk of development of neonatal lupus erythematosus (NLE).[2,11,12] NLE is an uncommon but life-threatening disease of the fetus and neonate, with important cardiac complications of CAVB, sinus bradycardia, QTc interval prolongation, endocardial fibroelastosis, and late-onset dilated cardiomyopathy.[3–5] Congenital CAVB develops in 1–5% of anti-Ro/SSA antibody-positive pregnancies, typically between 18 and 24 weeks of gestational age. Predominantly untreated CAVB has a mortality rate of 14–34%,[1–9] consistent with the untreated CAVB mortality rate of 26% in the current study.

The association of NLE with maternal anti-Ro/SSA antibodies is well established, but the trigger of the maternal antibody interaction with the fetal Ro particle is unclear in some antibody-exposed babies. The percentage of maternal anti-Ro/SSA antibody-positive fetuses with CAVB diagnosed in utero is unknown. Brucato et al and Jaeggi et al found maternal anti-Ro/SSA antibodies in 92% of 37 CAVB cases,[7,9] whereas in the present study maternal anti-Ro/SSA antibodies were detected in only 66% of 61 CAVB fetuses with a structurally normal heart. Jaeggi et al also reported that CAVB occurred in 5% of prospectively screened pregnancies with anti-Ro/SSA ELISA levels >100 U/ml, but did not occur in pregnancies with levels <50 U/ml.[6] Approximately two-thirds of anti-Ro/SSA antibody-positive mothers had low anti-Ro/SSA levels and probably little risk of development of fetal cardiac NLE.[8] It is unclear why the anti-Ro/SSA-positive rate in the present study was lower than in other reports. It is unlikely to be due to the sensitivity of the laboratory methods, but it is possible that other undetectable antibodies associated with congenital AVB are present in the Japanese population. Brucato et al and Lopes et al found similar mortality rates in the anti-Ro/SSA-positive and -negative groups,[7,8] and in the present multivariate analysis anti-Ro/SSA antibodies were not associated with prognosis.

Benefits and Risks of Transplacental Treatment

Congenital AVB is a progressively developing disease that evolves through 2 fundamental phases: an early phase characterized by the occurrence of still reversible AV conduction abnormalities (first- or second-degree AVB) and a final phase in which development of irreversible damage of the conduction system leads to the appearance of CAVB.[29] The specific pathogenetic mechanisms involved in the 2 phases have not been clarified, but there are 2 main theories. The first is based on an inflammatory-driven injury elicited by interaction between anti-Ro/SSA antibodies and specific antigens expressed in the conduction tissue of the fetal heart (inflammatory theory). The second theory involves electrophysiologic interference of anti-Ro/SSA antibodies with heart conduction (electrophysiological theory).[20] Consistent with these respective theories, steroids and i.v. immunoglobulins are used for anti-inflammatory treatment, while β-sympathomimetics are given for fetal pacing.

Several studies have found that a ventricular heart rate <55 beats/min is a risk factor for fetal and neonatal death,[4,14] and have recommended transplacental treatment with β-sympathomimetics to increase the heart rate. Jaeggi et al and Maeno et al, however, found that fetuses with CAVB without CHD and with a ventricular heart rate of <55 beats/min were not at risk.[30,31] In the present study, fetal ventricular heart rate did not influence fetal hydrops and prognosis, but treatment with a β-sympathomimetic agent was significantly associated with improved bradycardia.

To date, evidence of clinical efficacy of transplacental treatment has been limited to cases of congenital AVB.[13–19] Jaeggi et al reported a significant improvement in the outcome of fetal CAVB simultaneously with the introduction of routine perinatal treatment guidelines in 1997.[9] Hutter et al obtained an improved survival rate of >90% by initiation of maternal high-dose dexamethasone at the time of CAVB detection and maintenance of this dose during pregnancy, with addition of β-sympathomimetics to keep the fetal heart rate above 55 beats/min.[21] It was also suggested that prolonged use of dexamethasone might render a fetus with congenital CAVB less likely to develop additional manifestations of cardiac NLE such as myocarditis, cardiomyopathy, and hydrops fetalis, thus improving the overall outcome. The present findings suggest that use of steroids might render the affected fetus less likely to develop fetal hydrops, but that the neonatal survival rate improved only to 79%. The reason for the relatively bad prognosis in the present study may have been the difference in the rate of fetal hydrops compared to the Hutter et al study (42% vs. 10%). Undetectable autoantibodies or virus infection may be related to the increased rate of fetal hydrops in the Japanese population. Furthermore, Hutter et al initiated maternal high-dose dexamethasone at the time of CAVB diagnosis, at a mean gestational age of 24 weeks. The mean age of diagnosis was similar in the present study, but mean gestational age at which steroids were started was 26 weeks. In addition, the percentage of steroids used in transplacental treatment was

lower in the present patients (71% vs. 95%). These findings suggest that sufficient steroid dose at an early stage is very important to prevent fetal hydrops and to improve prognosis.

Use of steroids is controversial because of the potential risks for the fetus and mother, including problems with fetal growth restriction, oligohydramnios, and neurological development. Animal models suggest that repeated antenatal steroid doses can interfere with the growth and development of the immature brain, and human studies suggest that antenatal and postnatal dexamethasone may negatively affect a child's neuropsychological development.[22–24] In contrast, Brucato et al found no negative effects on neuropsychological development and intelligence in a cohort of preschool- and school-age children with CAVB who had been prenatally exposed to maternal anti-Ro antibodies and prolonged dexamethasone treatment.[25] The association of fetal growth restriction and oligohydramnios with antenatal steroids is well established, but the amount and length of steroid treatment that can be used safely is unclear. We note that development of fetal growth restriction and oligohydramnios are dose-related complications of steroids. Consequently, we recommend limiting steroid use to <10 weeks to avoid maternal and fetal adverse effects.

Prevention of Progression to Congenital CAVB

There are many case reports describing prevention of congenital CAVB, and first- or second-degree AVB is also relatively common and often normalizes spontaneously before or soon after delivery.[32] Recent prospective studies suggest that steroids and i.v. immunoglobulins are not beneficial for preventing progression to congenital AVB.[33,34] Similarly, the present study found a lack of superiority of transplacental treatment for second-degree AVB with bradyarrhythmia.

LQTS

Recent evidence has shown that anti-Ro/SSA antibodies are associated with prolongation of the QTc interval.[35] Although the exact arrhythmogenic mechanisms have not been clarified, anti-Ro/SSA antibodies may trigger rhythm disturbances through inhibition of cross-reactions with several cardiac ionic channels, including calcium channels and the hERG potassium channel.[36,37] Beta-sympathomimetics may trigger life-threatening arrhythmia such as VT/TdP in patients with LQTS, and therefore use of these drugs should be avoided in fetuses with QTc interval prolongation.[38,39] In the present study, in 4 of the 9 LQTS cases, emergency cesarean section was performed because of fetal VT/TdP at 33–36 weeks of gestational age. Oka et al also recently described atrioventricular block-induced TdP.[40] With this background, we recommend avoidance of β-sympathomimetics in a fetus with a heart rate >55 beats/min. Furthermore, assessment of QTc interval prolongation on magnetocardiography may be required to evaluate the risk of fetal congenital bradyarrhythmia.

Study Limitations

There were several limitations in the present study due to retrospective data selection bias and the relatively small sample size. The nature of a multicenter retrospective observational study using a questionnaire is such that the clinical data obtained vary among cases, so treatment bias may exist. Only ritodrine hydrochloride was used as β-sympathomimetic treatment, but was given in cases involving fetal heart rate >55 beats/min at some institutions, while dexamethasone, betamethasone and prednisolone were used as steroids at different doses among institutions. The follow-up period after birth was insufficient to permit analysis of long-term morbidity and mortality, and this prevented evaluation of potential long-term benefits and risks of transplacental medication. Finally, the sample size might have been too small to detect the effects of steroids on fetal congenital bradyarrhythmia. The steroid effect may become significant in a study with a higher number of cases.

Guidelines are required for transplacental treatment of fetal congenital bradyarrhythmia and follow-up after birth. We expect to analyze long-term outcome of fetal congenital bradyarrhythmia in a future study. Further large prospective studies are also needed to establish the most appropriate treatment strategies in Japan.

Conclusion

Beta-sympathomimetics improved bradycardia, but survival rate did not differ significantly in fetuses treated with and without transplacental medication. We recommend limiting steroid use to <10 weeks to avoid maternal and fetal adverse effects, with fetal growth restriction and oligohydramnios being of particular concern.

Acknowledgments

This work was supported by a grant from The Ministry of Health, Labor and Welfare of Japan (Health and Labour Science Research Grants for Clinical Research for New Medicine).

Disclosures

Sources of financial support: The Ministry of Health, Labor and Welfare of Japan (Health and Labour Science Research Grants for Clinical Research for New Medicine). None of the authors have a conflict of interest to disclose

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Anti-Ro/SSA Antibodies Are an Independent Factor Associated with an Insufficient Response to Tumor Necrosis Factor Inhibitors in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To study the significance of anti-Ro/SSA antibodies (anti-Ro) in the clinical response to tumor necrosis factor (TNF) inhibitors in patients with rheumatoid arthritis (RA).

Methods. The clinical responses of a cohort of 190 patients with RA who were treated with infliximab, etanercept, or adalimumab (n = 112, 64, and 14, respectively) as the first biologics were examined using the Disease Activity Score in 28 joints (DAS28) at 24 weeks and the discontinuation rate at 56 weeks. The baseline characteristics of responders and the nonresponders were compared. The clinical response was compared between anti-Ro-negative and -positive patients. The factors associated with the inefficiency of TNF inhibitors were estimated with a multivariable logistic regression analysis.

Results. The positive rate of anti-Ro was significantly higher in patients with no European League Against Rheumatism (EULAR) response at 24 weeks (OR 3.64, 95% CI 1.45–9.01, p = 0.003). In anti-Ro-positive patients, a moderate or good EULAR response rate was significantly lower with a sustaining higher median DAS28 (p = 0.006), and this difference was greater among infliximab-treated patients. The discontinuation rate for TNF inhibitors due to inefficacy at 56 weeks was also higher in anti-Ro-positive patients (OR 4.68, 95% CI 1.82–11.99, p = 0.0005), and 75% of these patients received infliximab. The presence of anti-Ro was strongly associated with no EULAR response at 24 weeks and a higher discontinuation rate of TNF inhibitors by 56 weeks (OR 5.22, 95% CI 1.75–15.57, p = 0.003 and OR 10.18, 95% CI 2.18–49.56, p = 0.003).

Conclusion. The presence of anti-Ro might be related to the lesser clinical response to infliximab compared to other TNF inhibitors, suggesting that the presence of anti-Ro should be considered when choosing the appropriate biologics for patients with RA. (J Rheumatol First Release Oct 1 2011; doi:10.3899/jrheum.101295)

Key Indexing Terms:
ANTI-RO/SSA ANTIBODIES TUMOR NECROSIS FACTOR INHIBITORS
RHEUMATOID ARTHRITIS AUTOANTIBODIES INFLIXIMAB

One of the crucial factors to consider when treating patients with rheumatoid arthritis (RA) is the presence of autoantibodies. It is well accepted that the anticyclic citrullinated peptide antibody (ACPA) is a prognostic factor for disease severity and radiographic progression in patients with RA[1,2,3,4]. Further, the production of autoantibodies, such as antinuclear antibodies (ANA) and anti-double stranded DNA antibodies (anti-dsDNA), is commonly observed in patients who have been treated with tumor necrosis factor (TNF) inhibitors, although these autoantibodies are induced at different rates for each TNF inhibitor[5,6,7].

Anti-Ro/SSA antibodies (anti-Ro) are frequently detected in rheumatic diseases such as Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), lupus-like condition, neonatal lupus erythematosus (NLE) and RA. The target antigen of anti-Ro consists of 2 different Ro proteins, 60 kDa and 52 kDa; and tissue injury in patients with NLE depends on the transplacental passage of these autoantibodies[8,9]. Anti-Ro is detected in 3% to 15% of patients with RA[10,11], and is associated with secondary SS, which is thought to be a clinically poor prognostic condition of RA[12]. However, anti-Ro also exists independently of SS, and the

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Supported by Japan Health and Labour Sciences Research Grants (grant no. H19-genomu-general-003).

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Accepted for publication July 8, 2011.

relationship between anti-Ro and the clinical features of RA have not been well studied.

We investigated the significance of anti-Ro in relation to the clinical response to TNF inhibitors in patients with RA. TNF inhibitors that were used as the first biologic disease-modifying antirheumatic drugs (DMARD) were less effective in anti-Ro-positive patients than in anti-Ro-negative patients. Moreover, multivariable logistic regression analysis demonstrated that anti-Ro was strongly associated with the inefficacy of TNF inhibitors in patients with RA.

MATERIALS AND METHODS

Patients. We examined a cohort of 190 Japanese patients with RA who visited Juntendo University Hospital, Tokyo, from October 2003 to May 2009 and were treated with one of the following TNF inhibitors as the first biologic DMARD: infliximab (IFX), etanercept (ETN), or adalimumab (ADA). All patients fulfilled the 1987 American College of Rheumatology classification criteria for RA[13]. Patients were diagnosed with secondary SS if they satisfied the following American-European consensus criteria for SS: the presence of ocular symptoms or oral symptoms plus any 2 from ocular signs, histopathology of the minor salivary gland, and salivary gland involvement[14]. Disease activity of RA was assessed by calculating the Disease Activity Score in 28 joints/C-reactive protein (DAS28/CRP). The clinical response rates at 24 and 56 weeks were compared between anti-Ro-positive and anti-Ro-negative patients with RA based on the DAS28 European League Against Rheumatism (EULAR) response criteria.

Antibody measurements. Anti-Ro and anti-La/SSB antibodies (anti-La) were measured using a double immunodiffusion test (DID) and precipitin reactions without serum dilutions were considered positive. Titers were determined by precipitin reactions with dilutions of serum (1:1 to 1:32). If the titer by DID was 1:32, a second assay was run, with serum diluted 1:64 to 1:2048. The prevalence of anti-Ro in healthy individuals as well as patients with SS, SLE, and scleroderma based on the DID assay was 0/100 (0%), 44/68 (64.7%), 28/57 (49.1%), and 4/22 (18.2%), respectively, which was comparable with previous reports[15,16,17,18]. Rheumatoid factor (RF) was measured by immunonephelometry, and levels > 20 IU/ml were considered positive. ACPA was detected using a second-generation ELISA (Mesacup; Medical & Biological Laboratories, Tokyo, Japan). The cutoff level for ACPA positivity was set at 4.5 arbitrary U/ml, and serum samples with ACPA levels above 200 arbitrary units were diluted further. ANA was tested using an indirect immunofluorescence assay on a fixed HEp-2 cell substrate, and levels ×20 were considered positive. Anti-dsDNA was measured using a radioimmunoassay, and levels > 6 IU/ml were considered positive. Serum samples were obtained from all patients before and 24 and 56 weeks after treatment and then stored at –20°C until used.

This study was approved by the Institutional Review Board at Juntendo University, and all patients provided written informed consent.

Statistical analysis. Continuous and categorical data are presented as the median and 25th–75th percentiles and counts or percentages, respectively. At the end of the study, differences in the following variables at baseline were compared between responders and nonresponders at 24 weeks and between patients with continuation and discontinuation of the TNF inhibitors at 56 weeks: sex, age, disease duration, methotrexate dose, steroid dose, previous DMARD, tender joint count, swollen joint count, global health using a 0–100 mm horizontal visual analog scale, modified Health Assessment Questionnaire (mHAQ), CRP levels, DAS28/CRP, IgG levels, ANA, anti-dsDNA, ACPA, RF levels, anti-Ro, anti-La, presence of secondary SS, and types of TNF inhibitors. The differences in these variables were also compared between the anti-Ro-negative and -positive groups. Categorical variables were analyzed using Fisher's exact test, while continuous variables were analyzed with the Mann-Whitney U test, and p values < 0.05 were considered statistically significant. The factors that were associated with a clinical response to the TNF inhibitors were assessed using a multivariable logistic regression analysis, and were used to estimate OR and their 95% CI. All the variables listed above were used to select the appropriate variables for the multivariable analysis using a backward stepwise method under the Akaike Information Criteria[19]. The goodness-of-fit of the model for the response variable vs the explanatory variables was evaluated based on the r-square value. Statistical analyses were performed using R version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org; 2008).

RESULTS

Baseline characteristics related to the clinical response to TNF inhibitors in patients with RA. Data from 188 patients who were treated with TNF inhibitors as a first biologic DMARD were analyzed. Two patients were withdrawn from the study because they had discontinued the TNF inhibitor by 24 weeks because of an infection. IFX, ETN, or ADA was administered to 112, 64, and 14 patients, respectively. Among these patients, 149 (79.3%) showed a moderate or good response at 24 weeks based on the DAS28 score. The baseline characteristics were compared between the responders and nonresponders at 24 weeks and between patients with continuation and discontinuation of the TNF inhibitors at 56 weeks (Table 1).

It was notable that the positive rate of anti-Ro and the prevalence of secondary SS at baseline were significantly higher in the nonresponders than the responders at 24 weeks (Table 1; OR 3.64, 95% CI 1.45–9.01, p = 0.003, and OR 2.68, 95% CI 0.99–6.98, p = 0.037, respectively). These measures were also significantly higher in patients with discontinuation of the TNF inhibitors at 56 weeks (Table 1; OR 4.68, 95% CI 1.82–11.99, p = 0.0005, and OR 3.35, 95% CI 1.21–8.94, p = 0.012, respectively). CRP and IgG levels were also higher in the nonresponders at 24 weeks (p = 0.008 and p = 0.006, respectively), but these were not statistically different between the responders and nonresponders at 56 weeks. Patients who had discontinued the TNF inhibitors at 56 weeks had a longer disease duration (p = 0.045).

Inefficiency of TNF inhibitors in anti-Ro-positive patients. We focused on the presence of anti-Ro and compared the baseline characteristics between anti-Ro-positive and anti-Ro-negative patients. There were no significant differences in sex, age, disease duration, or disease activity between these patient groups (Table 2). The prevalence of secondary SS was significantly higher in anti-Ro-positive patients than anti-Ro-negative patients (OR 30.09, 95% CI 10.29–98.00, p < 0.0001). Regarding serological factors, serum IgG was higher (p = 0.003) and anti-dsDNA and anti-La were detected more frequently in anti-Ro-positive patients (OR 9.27, 95% CI 1.69–63.23, p = 0.004, and OR infinity, 95% CI 2.12–infinity, p = 0.004, respectively), while the RF levels and the positive rate of ACPA were not significantly different between the 2 patient groups. When the clinical efficacy of the TNF inhibitors was compared at 24 weeks, the per-