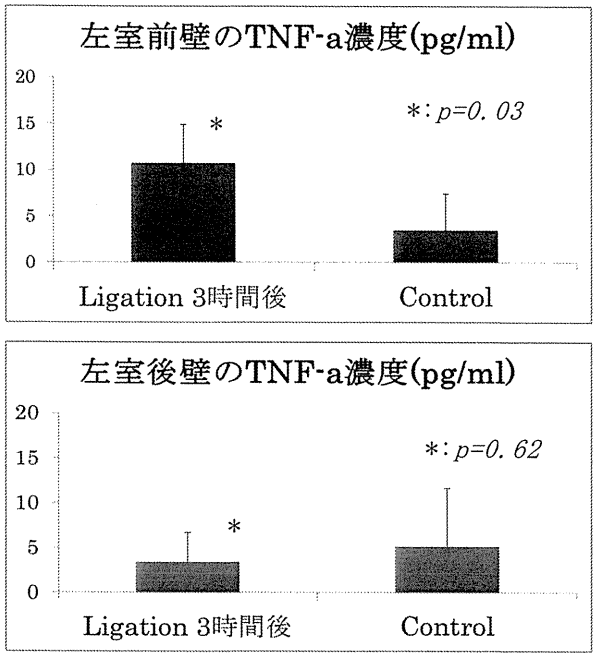
（図 1）虚血再灌流後 3 時間の心筋内 TNF-a 値



D. 考察

現在研究は途中段階ではあるが、心筋虚血再灌流モデルにおける全身炎症は確認出来ていない。また心筋内の炎症は、虚血心筋(前壁)において捉えられたが、程度としては極端に高いものではなかった。今後 Ligation の時間、組織採取のタイミングを変えて研究を進めていく必要があると考えられる。さらに MSC 移植によりこれらの炎症がどの程度抑えられるのか、効果時間はどの程度か、移植細胞数により効果が増減するのかを、検討していく予定である。

E. 結論

心筋虚血再灌流モデルにおいて、心筋局所の炎症が有意に起こっていることが確認できた。今後は MSC 移植による効果を検討していく。

G. 研究発表

1. 論文発表

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2) Marui A, Kimura T, Sakata R, 他 8 名 1,11 番目. Coronary revascularization in patients with liver cirrhosis. Ann Thorac Surg. 2011 May;91(5):1393-9.

3) Yanagi S, Matsumura K, Marui A, Morishima M, MD, Hyon SH, Ikeda T, Sakata R
Oral pretreatment with a green tea polyphenol for cardioprotection against ischemia - reperfusion injury in an isolated rat heart model.
J Thorac Cardiovasc Surg. 2011 Feb; 141(2): 511-7.

2. 学会発表

なし

H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3, その他

なし

＜実験 2：ラット人工心肺モデルに対する卵膜由来間葉系幹細胞の抗炎症療法の研究＞

A. 研究目的

高齢化および食生活の欧米化に伴い心血管病の罹患数は年々増加しており、心臓外科手術も多様化、複雑化傾向にある。とくに術前より高齢・糖尿病・慢性腎障害(CKD)・低心機能に伴う全身臓器の予備能低下を合併している場合は、人工心肺を用いた手術を行うことにより術後各種臓器障害から多臓器不全に陥る症例が少なくない。

これらの病態には、各種臓器への虚血再灌流障害と、全身性の強い炎症反応が共通している。特に体外循環においては、回路など人工表面と血液との接触による活性化、組織傷害（特に虚血再灌流障害)、エンドトキシン血症などの刺激により補体系を初めとした様々な生理活性が亢進し、好中球、マクロファージ、リンパ球などが活性化され、腫瘍壊死因子 tumor necrosis factor(TNF)やインターロイキン(IL)-1, 6, 8やプロスタグランジン(PGE)などの液性因子の産生が促進されることで、さらに組織損傷を来たすことが判明している。この全身性炎症反応を減弱するために、抗サイトカイン療法を初め様々な方法が試されてきたが、臨床の場で確実に効果が証明されているものはほとんどないのが現状である。

今回われわれは、卵膜由来間葉系幹細胞が持つ「抗炎症効果」に注目し、人工心肺に伴う強い全身性の炎症をどの程度制御できるかを検討するために、本研究の着想に至った。

B. 研究方法

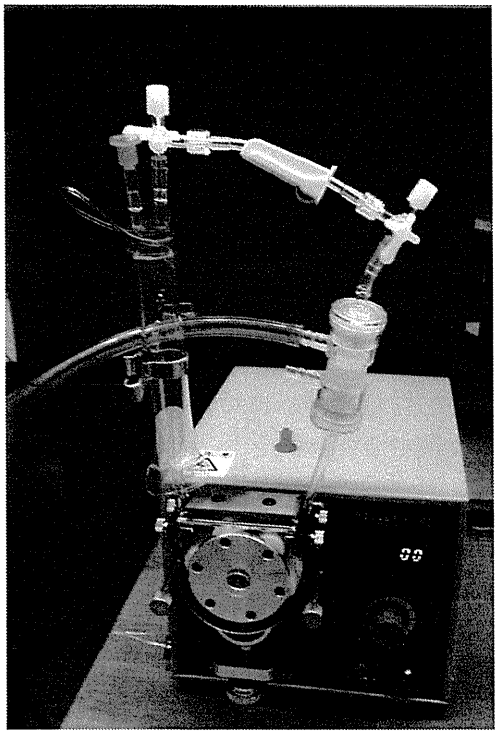
まず動物モデルを作成するにあたり、ラット用の人工心肺回路を独自に開発、作成(図 1)し、至適条件を詳細に設定した。

Lewis ラット(350-450g)に気管内挿管を行い人工呼吸麻酔管理とし、右大腿動脈に動脈圧測定用の留置針(24G)を、左大腿動脈に送血用の留置針(24G)を、右頸動脈より右心房に脱血用の留置針(17G)を挿入する。MSC 移植群ではこのタイミングで、ACI ラット卵膜より分離・培養した MSC を経静脈に移植する。

人工心肺のプライミングの後、50ml/min/kg の流量で 30 分間、人工心肺を回す。人工心肺中、mean blood pressure を 60mmHg 以上に保ち、直腸温を 36-37℃に保った。

15 分おきに血液ガスデータを採取して酸素化などのパラメータを確認した。

30 分の人工心肺の後、weaning して送血管、脱血管を抜去し、1 時間後に犠牲死として脾臓、肺、心臓、血清の採取を行った。

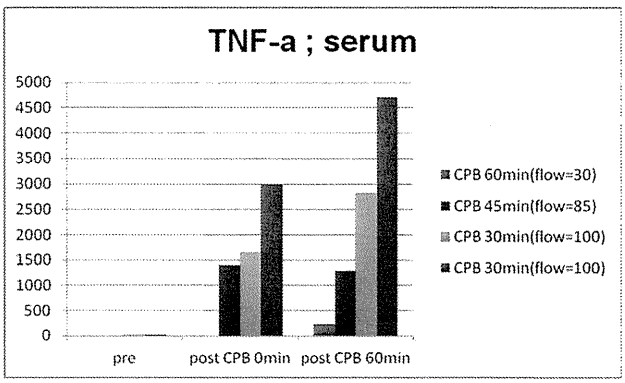


（図 1）オリジナルに作成したラット用人工心肺回路装置

C. 研究結果

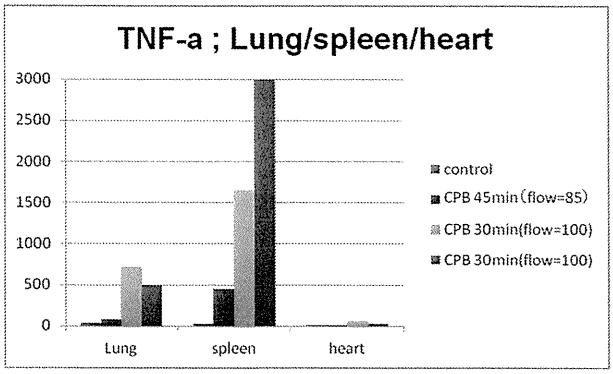
今回の至適条件に達するまでのデータを以下に示す。

血清中の炎症性サイトカイン(TNF-a)値は、人工心肺の時間よりも flow rate に依存して上昇する傾向にあり、その値は人工心肺前に比し、極めて高い値となった。（図 2）



（図 2）人工心肺の各種条件による、炎症性サイトカイン(TNF-a)上昇の相違

また、同様に高い flow rate では肺、脾臓での強い炎症が起こっていた。(図 3)



(図 3) 人工心肺後の肺、脾臓、心臓における炎症性サイトカイン(TNF-a)濃度

以上の結果および人工心肺中の血液ガスの結果から、50ml/min/kg での flow で 30 分間回す条件に決定し、現在 MSC 移植群の作成も含めて研究の遂行途中である。

D. 考察

われわれが独自に開発したラット用人工心肺回路を用いて、ラットモデルにおいて全身性に強い炎症が起こっていることが確認できた。至適条件の決定から、今後 MSC 治療群と MSC 非治療群での炎症の程度を確認するとともに、組織学的評価、MSC が炎症を制御するメカニズムの解析まで行っていく予定である。

E. 結論

ラット人工心肺モデルにおいて、人工心肺直後から強い炎症が起こっており、その値は flow rate に応じて上昇する傾向であった。

G. 研究発表

なし

H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3, その他

なし

厚生労働科学研究費補助金（免疫アレルギー疾患等予防・治療研究事業）
分担研究報告書

卵膜由来間葉系幹細胞よる炎症性消化器疾患に対する新規治療法の開発

研究分担者　大西　俊介　北海道大学大学院医学研究科助教

研究要旨

難治性の炎症性消化器疾患に対する新規治療法の開発を目的として，炎症性腸疾患，放射線腸炎，急性膵炎モデルラットを作成・確立し，同種卵膜由来間葉系幹細胞の投与による改善効果を検討した．炎症性腸疾患および放射線腸炎に対して改善傾向が認められた．

A．研究目的

炎症性腸疾患，放射線腸炎，急性膵炎など，しばしば重篤な経過をたどる炎症性消化器疾患に対する新規治療法のかい卵膜由来間葉系幹細胞の効果およびその機序を明らかにする．

B．研究方法

潰瘍性大腸炎モデルはACIラットに対し4%デキストラン硫酸(DSS)の7日間連続経口投与により作成した．2日目に5×10^5個のLewisラット由来卵膜由来間葉系幹細胞を静注し，7日後に病理学的方法等により効果判定した．

放射線腸炎モデルは腹部への8Gyの放射線照射により作成し，同日に5×10^5個のLewisラット由来間葉系幹細胞を静注して5日後に病理学的方法等により効果判定した．

急性膵炎モデルは肝門部胆管をクリッピングした後に3%タウロコール酸(TCA)を胆膵管内に逆行性に注入することで作成を試みた．

（倫理面への配慮）

ヒト卵膜由来間葉系幹細胞の分離・培養法を確立するため，ヒト卵膜を採取して動物実験に用いることについて，北海道大学大学院医学研究科で研究倫理審査中である。

C．研究結果

DSS腸炎モデルについては，間葉系幹細胞の投与によって体重減少の程度や大腸の長さ（図１），局所での炎症性サイトカインの発現において改善傾向を認めた．

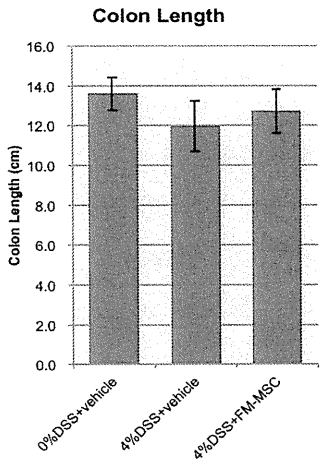


図１：DSS腸炎モデルラットの大腸の長さに対する卵膜由来間葉系幹細胞の効果

放射線腸炎モデルについては，体重減少（図２）や病理学的評価（図３）において改善傾向を認めた．

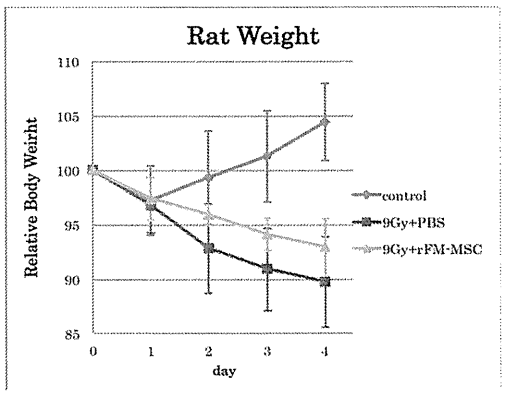


図２：放射線腸炎モデルラットの体重減少に対する卵膜由来間葉系幹細胞の効果

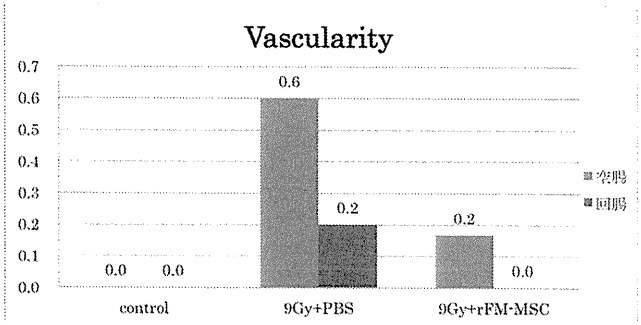


図３：放射線腸炎モデルラットの小腸血管密度に対する卵膜由来間葉系幹細胞の効果

急性膵炎モデルについては，TCAの注入により，ときに致死的な急性膵炎を発症したが，注入の際の漏れなどにより全く膵炎を発症しない場合も認められた.

D．考察

3種の炎症性消化器疾患モデルを作成し，そのうちDSS腸炎と放射線腸炎に対しては卵膜由来間葉系幹細胞の効果が認められる傾向にあったが，個体間のばらつきが多く，個体数を増やして再検討するべきと考えられた．さらに，DSSの投与量や放射線照射量を複数設定する必要もあると考えられた．また，急性膵炎モデルについては，手技的な問題が大きかったため，TCAの胆膵管内注入の精度を上げる必要があると考えられた.

E．結論

3種類の炎症性消化器疾患のモデルを作成し，その効果を観察したが，十分とは言えなかった．今後投与法等について検討が必要である.

F．健康危険情報

該当なし

G．研究発表

1.　論文発表

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2.　学会発表

該当なし

（発表誌名巻号・頁・発行年等も記入）

H．知的財産権の出願・登録状況

（予定を含む。）

1. 特許取得

該当なし

2. 実用新案登録

該当なし

3. その他

該当なし

厚生労働科学研究費補助金（免疫アレルギー疾患等予防・治療研究事業）

分担研究報告書

「卵膜由来間葉系幹細胞によるT細胞分化制御に関する研究」

研究分担者　名前　山原　研一　　所属　国立循環器病研究センター

研究要旨：卵膜MSCによる免疫調節効果を、従来検討されてきた骨髄MSCとの比較において、T細胞分化の観点から解析を行った。Th1およびTh17分化誘導系において、卵膜MSCは骨髄MSC以上にTh1およびTh17の増殖および分化を抑制した。卵膜MSCによる免疫調節機構に、Th1およびTh17を介した作用が含まれることを証明した。

A．研究目的

近年、組織再生を目的とした研究が進んできた間葉系幹細胞（MSC）であるが、最近はその免疫調節作用が注目され、骨髄移植におけるGVHD予防では臨床応用研究が開始されている(Lancet. 371:1579-86,2008)。しかしながら、組織再生同様、骨髄MSCを中心とした研究が主体であり、卵膜MSCの免疫制御の可能性を考慮した研究は進んでいない。特に卵膜MSCは他家移植が想定されるため、骨髄などの自己MSCと比較し、ホスト側の免疫反応により治療効果が異なる可能性が考えられる。しかしながら、卵膜を含む胎児付属物は免疫原性が低いことから(Circulation 112:214-23,2005)、他家卵膜MSC移植は自己骨髄MSC同様の免疫調節効果を示す可能性がある。そこで、卵膜MSCによる免疫調節効果を、従来検討されてきた骨髄MSCとの比較において、T細胞分化の観点から解析を行った。

B．研究方法

Tリンパ球分化における卵膜MSCによる効果検討

Th1およびTh17分化誘導におけるMSCの効果を検証するため、当センターにて分離したヒト卵膜MSC、市販ヒト骨髄MSC、及び市販ヒトCD4陽性Tリンパ球を用いたin vitro実験を行った。即ち、ヒトCD4陽性T細胞($5x10^4$)に(a)Th1誘導：抗CD28抗体、抗CD3抗体、抗IL-4抗体、IL-2、IL-12を添加、(b)Th17誘導：抗CD28抗体、抗CD3抗体、TGF-b、IL-1b、IL-6、IL-23を添加し、同時にヒト卵膜MSCあるいはヒト骨髄MSC（$5x10^5$）との共培養にて5日間培養した。その後、浮遊しているT細胞数を計測し、更にFACSにて(a)IFN-γ・CD4陽性Th1細胞、(b)IL-17・CD4陽性Th17細胞を計測した。

（倫理面への配慮）

ヒト胎児付属物の採取は、「臨床研究に関する倫理指針」を遵守し、当センターの倫理委員会の審査により承認後、提供者本人による書面での同意を得て実施した。また、提供者に対するインフォームド・コンセントは書面で行った。

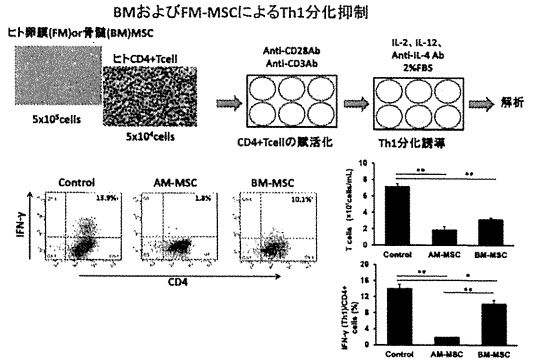
C．研究結果

Tリンパ球分化における卵膜MSCによる効果検討

(a)Th1誘導

Th1誘導下において、T細胞数は$7.1\pm0.4\times10^5$に、IFNγ陽性Th1細胞は

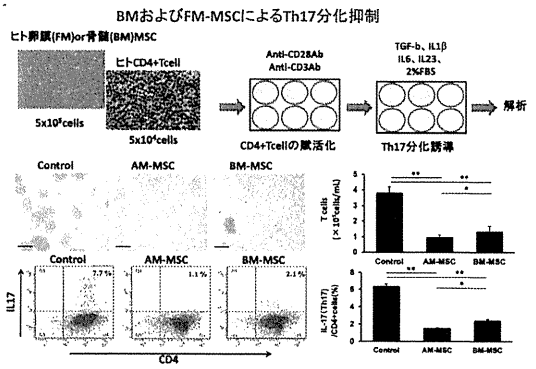
(13.9±1.2%)まで増加した。一方卵膜あるいは骨髄 MSC と共培養した場合、T 細胞数はそれぞれ $1.8\pm0.4\times10^5$ と $3.1\pm0.3\times10^5$、Th1 細胞は 1.8±0.2 % および 10.1±1.0 % (P<0.01)まで著名に抑制されていた（図１）。



（図１）骨髄 (BM) MSC および卵膜 (FM) MSC による Th1 分化誘導抑制

(b)Th17 誘導

Th17 誘導では、T 細胞数は $3.8\pm1.6\times10^5$ に、IL-17 陽性 Th17 細胞は 6.8±0.4%となった。卵膜あるいは骨髄 MSC の共培養により、Th1 誘導同様、T 細胞数はそれぞれ $0.9\pm0.6\times10^5$ と $1.3\pm1.5\times10^5$(P<0.01)、Th17 細胞は 1.5±0.1%および 2.3±0.3%(P<0.01)まで著名に抑制されていた。



（図２）骨髄 (BM) MSC および卵膜 (FM) MSC による Th17 分化誘導抑制

考察

本研究では、卵膜 MSC による免疫調節作用について、骨髄 MSC と比較しながら、T 細胞との共培養により検討を行った。

我々の検討結果から、Th1 分化および Th17 分化いずれの実験系においても、卵膜 MSC はその増殖、および、誘導を著明に抑制した。予想外なことに、この効果は骨髄 MSC と比較すると卵膜 MSC においてより強力なものであった。このことは、卵膜 MSC が骨髄 MSC よりもより強力な免疫抑制効果を有していることを示唆する所見であり、現在その検証を行っているところである。特に MSC による免疫調整作用は、これまで液性因子を介した検討がされてきており、我々も液性因子にターゲットを絞り研究を進めている。

E．結論

卵膜 MSC による免疫調節効果を、従来検討されてきた骨髄 MSC との比較において、Th1 および Th17 分化に注目して検討した。結果、Th1 および Th17 分化誘導系において、細胞増殖および分化を卵膜 MSC は著明に抑制し、その効果は骨髄 MSC よりも強力であった。

G．研究発表

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H．知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
なし

2. 実用新案登録
なし

3.その他
なし

Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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雑誌

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Ⅳ. 研究成果の刊行物・別刷

Circulation Journal
Official Journal of the Japanese Circulation Society
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ORIGINAL ARTICLE
Aortic Disease

Pregnancy-Associated Aortic Dilatation or Dissection in Japanese Women With Marfan Syndrome

Shinji Katsuragi, MD; Keiko Ueda, MD; Kaoru Yamanaka, MD; Reiko Neki, MD; Chizuko Kamiya, MD; Yoshihito Sasaki, MD; Kazuhiro Osato, MD; Koichiro Niwa, MD; Tomoaki Ikeda, MD

Background: Aortic dilatation and dissection are severe complications of pregnancy that may cause maternal death. The purpose of the present study was to investigate risk factors for aortic dilatation or dissection in pregnant Japanese women with Marfan syndrome.

Methods and Results: A total of 28 patients with Marfan syndrome were investigated retrospectively during pregnancy and after delivery at 1 institution. These patients were divided into 2 groups: those who experienced aortic dilatation or dissection (group D, n=11) and those who did not (group ND, n=17). In group D, aortic dilatation or dissection occurred in 7 cases during pregnancy (2 in the 2nd trimester, 5 in the 3rd trimester) and 4 cases after birth. The 2 cases in the 2nd trimester involved aortic dilatation >60 mm and those patients underwent hemiarch replacement and a David operation, respectively. Delivery by cesarean section (64% vs. 18%, P<0.05), sinus of Valsalva ≥40 mm (86% vs. 21%, P<0.05), aortic size index (size of sinus of Valsalva/body surface area) ≥25 mm/m^2 (7/7, 100% vs. 0/14, 0%, P<0.0001), and faster growth of the sinus of the Valsalva (median, [interquartile range]: 0.41 mm/month [0.23–0.66 mm/month] vs. 0.05 mm/month [–0.13 to 0.22 mm/month]; P<0.05) were significantly higher in group D than in group ND.

Conclusions: A large sinus of Valsalva, increased aortic size index, and rapid growth of the sinus of Valsalva are risk factors for aortic dilatation or dissection in pregnant Japanese women with Marfan syndrome. (*Circ J* 2011; **75:** 2545–2551)

Key Words: Aortic dissection; Aortic size index; Marfan syndrome; Pregnancy; Sinus of Valsalva

Marfan syndrome is an autosomal dominant connective tissue disorder caused by mutations in the fibrillin-1 (FBN1) gene located on chromosome 15.[1] These mutations result in weakness of the supportive tissue of the body, and clinical characteristics include symptoms of the cardiovascular, skeletal, and ocular systems.[2,3] Cardiovascular complications are the main cause of morbidity and mortality in patients with Marfan syndrome.[4] Before the development of preventive surgical approaches to aortic diseases, the mean life expectancy for a patient with Marfan syndrome was <40 years, with aortic dissection, aortic rupture and cardiac failure being the predominant causes of death.[5] Beta-blocker therapy and elective surgical repair, however, have increased life expectancy to near normal values.[6]

Editorial p2532

Pregnancy is strongly associated with life-threatening problems in Marfan patients. The risk of aortic dilatation or dissection increases during and after pregnancy in patients with Marfan syndrome due to superimposition of the hyperdynamic and hypervolemic circulatory state of pregnancy on the preexisting weakness of the aortic media.[3] The rate of aortic dissection during pregnancy has been studied in previous reports. In 1981, Pyeritz reported no aortic complications during 105 pregnancies in 26 women affected by Marfan syndrome, based on phone interviews.[7] Rossiter et al prospectively followed 45 pregnancies in 21 women, and found 2 cases complicated by dissection;[8] Lipscomb et al reported 6 aortic events, including 4 aortic dissections, in 91 pregnancies in 36 women;[9] Lind and Wallenburg found 5 aortic dissections in 117 pregnancies;[10] and Pacini et al reported 7 aortic dissections in 160 pregnancies in 85 women.[11] Combining all these data gives a risk of 3.9% for aortic complication during pregnancy in women with Marfan syndrome who are not taking β-blockers.

The indicators of aortic risk in pregnancy are an aortic diam-

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Table 1. Patient Features vs. Presence of Aortic Dilatation or Dissection

	Group D (n=11)	Group ND (n=17)	P value
Maternal age (years)†	29.5±3.5	30.1±4.0	NS
Height (cm)†	167.3±4.5	165.3±4.2	NS
Weight (kg)†	65.3±4.5	64.5±3.9	NS
Nulli/Multiparous‡	8/3	12/5	NS
Gestation (weeks)†	36.2±3.2	37.0±2.8	NS
Birth weight (g)†	2747±705	2769±599	NS
Delivery mode‡			<0.005
Vaginal delivery	4	14	
Cesarean section	7	3	
BMI†	24.2±1.5	24.1±1.8	NS
DM‡	2	3	NS
Hypertension‡	2	3	NS
Smoking‡	2	3	NS

Data given as n or mean±SD. †Student's t-test; ‡chi-square test and Fisher's exact test. P<0.05, significant difference. D, aortic dilatation or dissection; ND, no aortic dilatation nor dissection; BMI, body mass index; DM, diabetes mellitus.

Table 2. Diagnostic Categories for Marfan Syndrome (Ghent Criteria[18])

Category	Group D (n=10)		Group ND (n=12)	
	Major	Minor	Major	Minor
Skeletal	10	3	11	2
Ocular	2*	1	8*	1
Cardiovascular	10*	3	7*	5
Pulmonary	–	3	–	2
Skin	–	0	–	1
Dura	2	–	5	–

Data were analyzed using chi-square test and Fisher's exact test.
*P<0.05.
Abbreviations see in Table 1.

eter ≥4.0 cm[7–10,12,13] and a steady increase in the aortic root dimension during pregnancy.[9,10,14] Meijboom et al reported that pregnancy in women with Marfan syndrome seems to be relatively safe up to an aortic root diameter of 45 mm.[15] Most previous reports on Marfan syndrome in pregnancy, however, have been from North America or Europe, and people in these areas have relatively large physiques, and patient physique was not standardized. Because normal aortic dimensions vary with age and body size,[16] the same aortic dimension represents a proportionally greater diameter in smaller individuals, and proper interpretation of the aortic dimension requires that age and body size are accounted for. Therefore the absolute aortic size cannot be directly used to evaluate risk in patients with a small physique,[17] such as Japanese women.

The risk factors for aortic complications in pregnant patients affected with Marfan syndrome have not been examined relative to body surface area. Therefore, to improve patient management, we studied 28 consecutive pregnant patients with Marfan syndrome in 1 institution to determine the factors that influence maternal aortic complications.

Methods

Patients

We retrospectively analyzed 28 consecutive pregnant patients with Marfan syndrome who were managed at the National Cerebral and Cardiovascular Center from 1991 to 2007. Diagnosis of Marfan syndrome was made based on the original Ghent criteria (1996).[18] Cases before 1996 were confirmed to fulfill these criteria. The initial assessment included an evaluation of personal history and detailed family history, and a clinical examination including ophthalmological tests and a transthoracic echocardiogram.[3] X-ray was used to detect protrusion acetabulae, and lumbar magnetic resonance imaging (MRI) was performed to detect dural ectasia.

We divided the patients into 2 groups: those with aortic dilatation or dissection (group D, n=11) during pregnancy or within 1 year after delivery and those without aortic dilatation or dissection (group ND, n=17). Aortic dilatation was defined as a diameter >60 mm at any part of the aorta.

Measurement of Aortic Diameter and Indication for Surgery

Measurement of the sinus of Valsalva was made on echocardiography in 2-D parasternal long-axis views at end-diastole using the leading edge to leading edge method.[16,19] MRI and computed tomography were not routinely used. The Japanese Circulation Society recommends an operation for patients with a sinus of Valsalva >5 cm (class IIa, level C) in all cases of Marfan syndrome.[20] Some surgeons also recommend an operation for patients with a sinus of Valsalva >4.5 cm.[21] At the National Cerebral and Cardiovascular Center, surgical intervention is indicated according to the aforementioned criteria and for patients with a family history of dissection or sudden death. In general, surgical intervention is indicated for a sinus of Valsalva >4.0 cm or in a case of steady aortic growth.[22,23] During pregnancy, surgical intervention is indicated if there is steady aortic growth or massive dissection. To standardize the measurement based on body size, we expressed the size of the sinus of Valsalva using the aortic size index (ASI), which is calculated as: ASI=aortic diameter (mm)/body surface area (m^2).[17]

Management During Pregnancy

Echocardiographic follow-up including aortic diameter measurement and Holter electrocardiogram was performed at least once in each trimester during pregnancy and within 4 weeks after delivery. When surgical intervention was indicated, the operation was performed after cesarean section in the case of a mature fetus. When the fetus was too immature to live independently, the operation was performed with the fetus in the uterus.

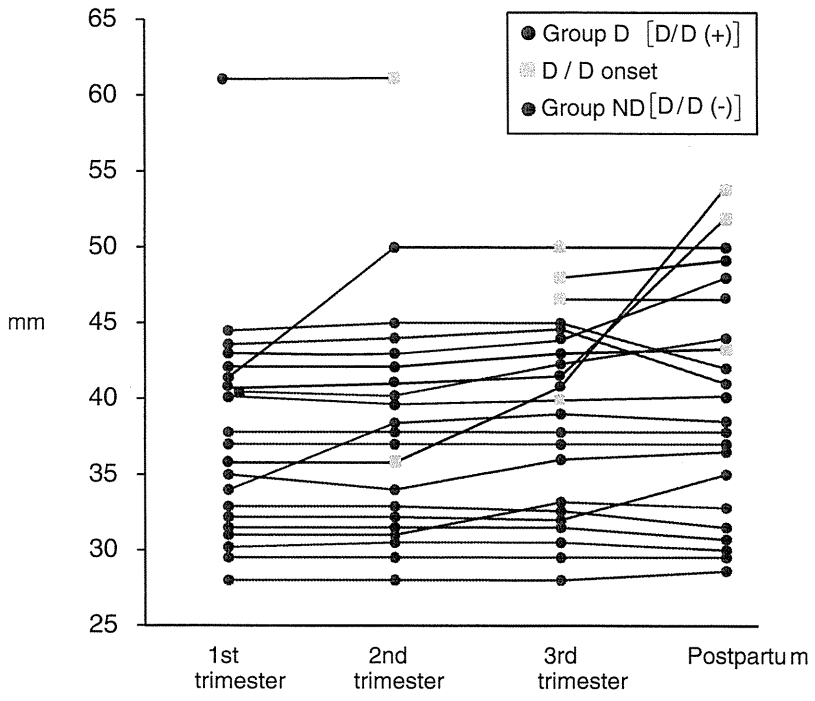

Figure 1. Diameters of the sinus of Valsalva. D/D, aortic dilatation or dissection. Red, presence of aortic dilatation or dissection; blue, absence of aortic dilatation or dissection (group D and group ND, respectively). Pink, cardiac event. In the 1st trimester a larger sinus of Valsalva was found in group D compared to group ND (41.5±2.4mm vs. 34.8±1.3mm P<0.05; Student's t-test). The sinus of Valsalva was ≥40mm in 6/7 patients in group D and 3/14 in group ND (P<0.05; chi-square test and Fisher's exact test).

Table 3. Echocardiographic Findings vs. Presence of Aortic Dilatation or Dissection

Item	Group D (n=11)	Group ND (n=17)	P value
Sinus of Valsalva (mm) in first trimester†	44.1±10.2	34.8±5.5	<0.005
Growth of aorta (mm/month)‡	0.41 (0.23–0.66)	0.05 (–0.13 to 0.22)	<0.005
Aortic valve regurgitation§			
None-Mild	5	15	<0.05
Moderate-Severe	6	2	
Mitral valve prolapse§	6	3	<0.05
LVDd†	45.8±7.1	44.8±6.8	NS
LVDs†	31.1±4.7	30.1±4.6	NS
%FS†	36.5±5.6	37.5±4.6	NS
RA cavity enlarged§	2	3	NS
RV cavity enlarged§	2	2	NS
PA dilatation (≥20mm)§	3	2	NS

Data given as mean±SD, n, or median (interquartile range).
†Student's t-test; ‡Wilcoxon test; §chi-square test and Fisher's exact test. P<0.05, significant difference.
LVDd, left ventricle end-diastolic dimension; LVDs, left ventricle end-systolic dimension; FS, fractional shortening; RA, right atrium; RV, right ventricle; PA, pulmonary artery. Other abbreviations see in Table 1.

Data Collection

Data were collected on family history (sudden death, aortic dilatation or dissection), maternal age, body height, body weight, parity, presence or absence of hypertension, diabetes mellitus, change in the diameter of the sinus of Valsalva during and after pregnancy, right and left ventricular function, aortic valve regurgitation, mitral valve prolapse, delivery mode (Cesarean section or vaginal delivery), time of delivery (gestational weeks), and birth weight.

Statistical Analysis

For continuous variables, Student's t-test was used for analysis of normally distributed data and the Wilcoxon test was used for data that were not normally distributed. A chi-squared test and a Fisher's exact test were used for comparing categorical variables between the 2 groups. All statistical analyses were performed using JMP 7 (SAS Institute, Cary, NC, USA). P<0.05 was considered statistically significant.

Results

Aortic Dilatation or Dissection Group

Eleven patients had aortic dilatation or dissection associated with pregnancy (in 7 this occurred during pregnancy and in 4 it occurred within 1 year after pregnancy). Two of the 7 antepartum cases involved aortic dilatation >60mm (maximum diameter of the aorta) in the 2nd trimester at 16 and 19 weeks of gestation, respectively. One patient underwent hemiarch

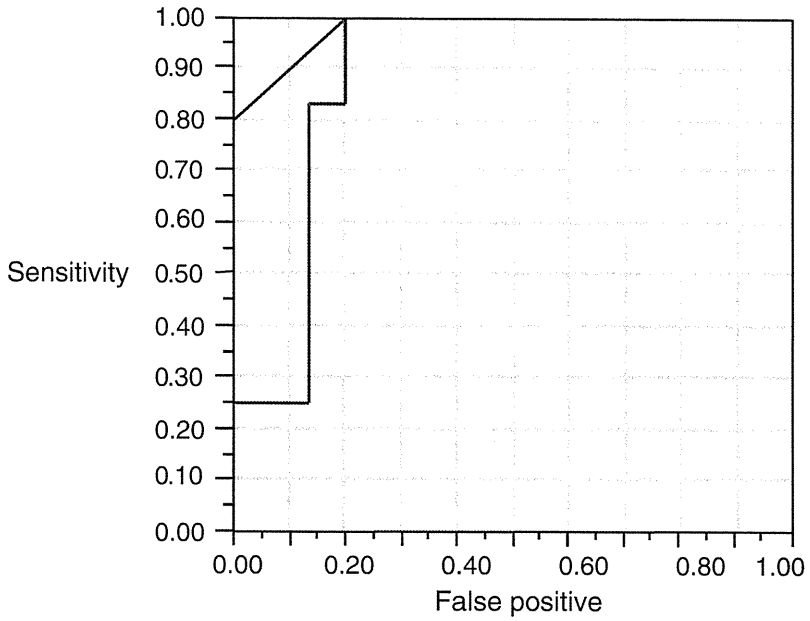

Figure 2. Receiver operating characteristic curve for the risk of dilatation or dissection based on the size of the sinus of Valsalva.

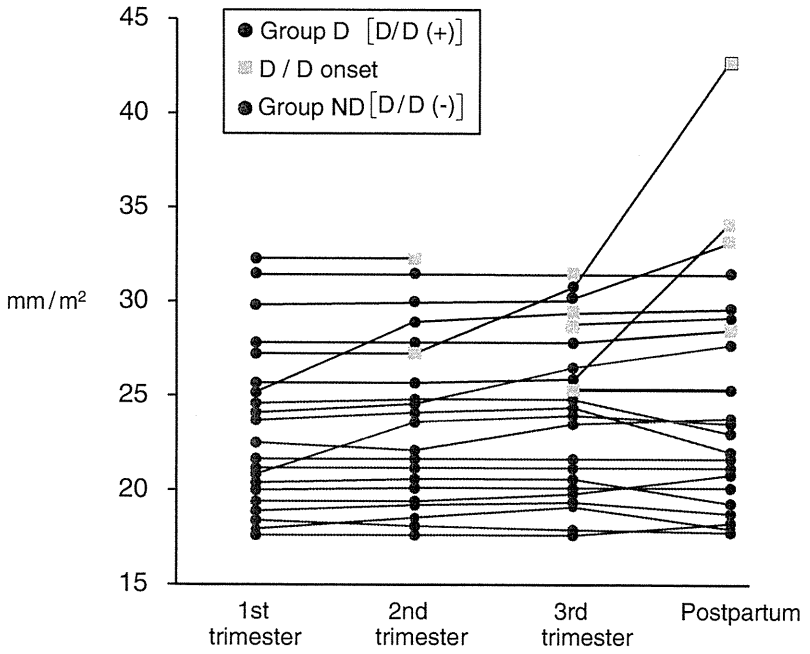

Figure 3. Sinus of Valsalva relative to body surface area (size of the sinus of Valsalva/body surface area: mm/m²). D/D, aortic dilatation or dissection. Red, presence of aortic dilatation or dissection; blue, absence of aortic dilatation or dissection (group D and group ND, respectively). In the 1st trimester the normalized size of the sinus of Valsalva was ≥25 mm/m² in 7/7 patients in group D and 0/14 in group ND ($P<0.0001$; chi-square test and Fisher's exact test).

replacement and the other underwent a David operation under cardiopulmonary bypass with the fetus in the uterus. The other 5 dissections during pregnancy occurred at 29, 33, 34, 35 and 39 weeks of gestation. Of these 5 patients, 3 underwent Bentall operations following cesarean section and 2 received conservative therapy after cesarean section. Dissection in the 4 postpartum cases occurred at 4 days, 8 months, 18 months, and 11 months after delivery, respectively. Of the 11 dilatation or dissection cases, 8 occurred in the ascending aorta, 1 in the descending aorta, and 2 in both locations.

Demographic Patient Data

The maternal age, week of delivery, and birth weight did not differ between the D and ND groups (**Table 1**). The incidence of cesarean section was higher in group D than in group ND (7/11, 63.6% vs. 3/17, 17.6%, $P<0.05$). This was attributed to performance of cesarean section due to occurrence of dilatation or dissection of the aorta during pregnancy. The number of patients meeting each diagnostic category for Marfan syndrome (Ghent criteria, 1996)[18] is given in **Table 2**. In group D, fewer patients met the major ocular criteria (2/10, 20% vs.

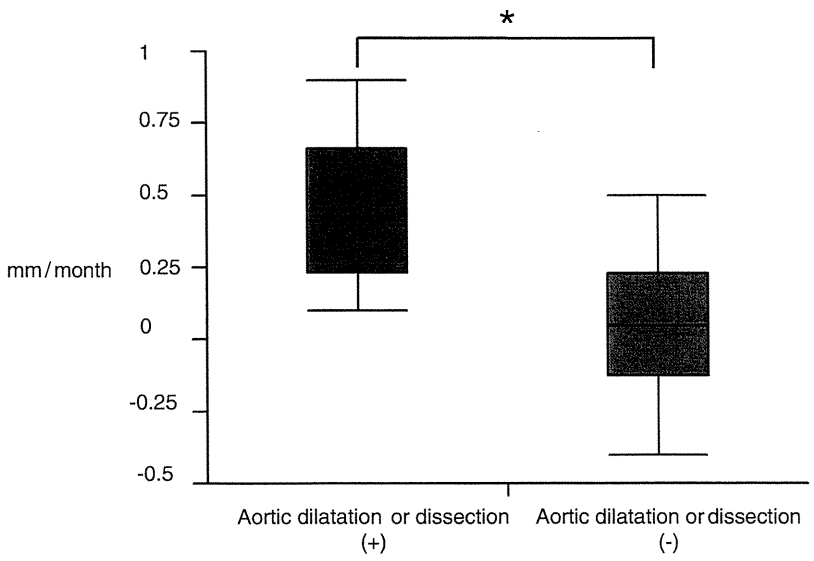

Figure 4. Rate of growth of the sinus of Valsalva. Significantly faster growth of the sinus of Valsalva was observed in group D (red) than in group ND (blue). The middle bar indicates the 50th percentile; box edges are the 25th and 75th percentiles; and outer bars are the 10th and 90th percentiles. Data were analyzed using Wilcoxon test. *P<0.05.

8/12, 67%, P<0.05) and more patients met the major cardiovascular criteria (10/10, 100% vs. 7/12, 58%, P<0.05). Gene analysis was performed in 11 of the 28 cases (40%) and a fibrillin-1 mutation was found more commonly in group D, although the difference was not significant (4/4, 100% vs. 4/7, 57%, P=0.06). A family history of sudden death or aortic dissection was more frequent in group D (7/11, 64% vs. 4/17, 24%, P<0.05).

Echocardiographic Patient Data

The sinus of Valsalva in the 1st trimester of pregnancy was significantly larger in group D than in group ND (mean [range]: 44.1 mm [36–61 mm] vs. 34.8 mm [28–45 mm], P<0.005; **Figure 1**; **Table 3**) and a sinus of Valsalva ≥40 mm in the 1st trimester was more frequent in group D (6/7, 86% vs. 3/14, 21%, P<0.05; **Figure 1**). On receiver operating characteristic (ROC) analysis of the relationship of the size of the sinus of Valsalva in the 1st trimester with aortic dilatation or dissection during pregnancy and after birth, the area under the curve (AUC) was 0.837 and the size of the sinus of the Valsalva that produced the best sensitivity (1–specificity) was 40 mm (**Figure 2**).

An ASI (diameter of the sinus of Valsalva/body surface area) ≥25 mm/m^2 was more frequent in group D than in group ND (7/7, 100% vs. 0/14, 0%; P<0.0001; **Figure 3**). On ROC analysis of the relationship of the ASI in the 1st trimester with aortic dilatation or dissection during pregnancy and after birth, the AUC was 0.985 and the size of the sinus of Valsalva that produced the best sensitivity (1–specificity) was 25 mm/m^2. In 1 case, aortic dissection occurred in a patient with a sinus of Valsalva of only 36 mm in the 1st trimester. Her ASI, however, was 27.3 mm/m^2 (36 mm/1.31 m^2), which was the 5th largest in the study. This indicates that normalizing the sinus of Valsalva measurement with respect to body surface area is more appropriate for prediction of aortic dilatation or dissection, compared to the absolute diameter. Significantly faster growth of the sinus of Valsalva was also observed in group D (median [interquartile range]: 0.41 mm/month [0.23–0.66 mm/month] vs. 0.05 mm/month [–0.13 to 0.22 mm/month]; P<0.05; **Figure 4**).

The sizes of the right and left ventricles did not differ between the 2 groups (**Table 3**). In the 1st trimester of pregnancy, patients in group D had more frequent moderate to severe aortic valve regurgitation (6/11, 55% vs. 2/17, 12%; P<0.05) and mitral valve regurgitation (6/11, 55% vs. 3/17, 18%; P<0.05). These effects were already present before conception and may be 1 of the causes of dilatation or dissection.

Discussion

This is the first study to investigate the risk factors for pregnancy-associated dilatation or dissection in Japanese patients with Marfan syndrome. The risk factors that differed significantly between groups D and ND were mostly consistent with those found in previous studies.[7–10,14] These factors included a large sinus of Valsalva, rapid growth of the sinus of Valsalva during pregnancy, moderate to severe aortic valve or mitral valve regurgitation, and a family history of sudden death or aortic dissection.

We found that a large sinus of Valsalva (≥40 mm) at the start of pregnancy was a risk factor for dilatation or dissection during pregnancy and after birth. The present result differs from the findings of the relatively large prospective study by Meijboom et al, in which it was concluded that pregnancy in women with Marfan syndrome seems to be relatively safe up to an aortic root diameter of 45 mm,[15] and from Canadian guidelines that recommend that women with an aortic root diameter beyond 44 mm should be strongly discouraged from becoming pregnant.[24] Taking into account that Japanese women have a generally smaller physique than European and North American women, we recommend that the cut-off for Japanese patients for advice regarding avoidance of pregnancy should be a sinus of Valsalva diameter ≥40 mm, rather than ≥45 mm. In a case report on a patient who developed a massive retrograde type B aortic dissection 7 days after normal spontaneous vaginal delivery, Gandhi et al described the patient as "petite" (body surface area, 1.69 m^2), but this is still larger than the average Japanese woman.[25]

We also suggest that normalizing the diameter of the sinus of Valsalva with regard to body surface area (diameter of the Valsalva/body surface area; mm/m^2) may be more appropriate

for detection of high-risk cases at the start of pregnancy. The relative aortic size was first used to predict complications in patients with thoracic aortic aneurysms.[17] We found that an ASI ≥25 mm/m^2 in the 1st trimester is associated with a high risk for aortic dilatation or dissection during pregnancy and after birth. The ASI is a novel measurement of relative aortic size that predicts rupture of aortic aneurysm,[17] and Davies et al found that the ASI was more important than absolute aortic size in predicting aortic complications, especially in smaller women such as those in the Japanese population.[17] We found that there was more rapid growth of the sinus of Valsalva in patients with Marfan syndrome with pregnancy-associated aortic dilatation or dissection, compared to those without these conditions. Therefore, even if the diameter of the sinus of the Valsalva is small, rapid growth carries a risk of aortic dissection or dilatation. The same phenomenon has been reported in non-pregnant cases of Marfan syndrome. Meijboom et al followed 108 women with Marfan syndrome and aortic root growth prospectively using serial echocardiograms, and found that the patients could be divided into 2 normally distributed groups based on aortic growth rates: 90% had slow growths and 10% had fast growth.[15] Significantly more dissections of the ascending aorta (25% vs. 4%, P<0.001) were observed in the fast growth group, and the average growth of the sinus of Valsalva in the fast group was 1.8 mm/year. The median growth in the present 5 cases of aortic dissection was as high as 4.1 mm/year. This large increase relative to that in the Meijboom et al study[15] is probably due to the maternal cardiovascular changes in pregnancy, including increased blood volume, heart rate, and stroke volume.[25] Furthermore, hormonally mediated histological changes also occur in the aorta, including a decrease in mucopolysaccharides and loss of elastic fibers in the aortic wall.[26–28] Care is therefore required in treating patients with a high growth rate of the sinus of Valsalva. The frequency and degree of aortic and mitral valve regurgitation were also higher in patients with aortic dilatation or dissection, and these valvular changes may have been the causes of dilatation or dissection.

An international expert panel established the revised Ghent criteria in 2010, which, first, focused more on cardiovascular manifestations, and in which aortic dilatation/dissection and ectopia lentis are the cardinal clinical features.[29] Second, in these revised criteria, a more prominent role is assigned to molecular genetic testing of FBN1 and other relevant genes in the diagnostic assessment. Third, some of the less specific manifestations of Marfan syndrome were either removed or made less influential in the diagnostic evaluation of patients. The new criteria also differentiate Marfan syndrome from Marfan-related syndromes such as Loeys-Dietz syndrome, Ehlers-Danlos syndrome, and familial thoracic aortic aneurysm syndrome, which are associated with a significantly greater risk of cardiovascular problems.[29–31] In the present study, patients with dilatation or dissection of the aorta were less likely to meet major ocular criteria, and more likely to meet the major cardiovascular criteria and had a more frequent family history of dilatation or dissection. These findings indicate that the new diagnostic criteria for Marfan syndrome facilitate identification of high-risk patients for pregnancy-associated dilatation or dissection more accurately.

Study Limitations

The disease severity of the present patients may have been higher than that of general Marfan syndrome patients because the National Cerebral and Cardiovascular Center is a referral center for cardiovascular diseases, and we also perform gene analysis.[32] Therefore, most Marfan syndrome patients are referred to our center due to cardiovascular complications and many have a family history of aortic complications. Also, because we investigated the clinical courses of Marfan syndrome patients associated with pregnancy in one institution, only 28 patients were included in the study. The small number of subjects prevented correction of the results for the effects of potential confounding factors such as hypertension, and we could not perform multifactorial analysis. The present study, however, has the advantage of clear definition of medical and surgical treatment and obstetric management. Measurements of the aorta, ventricle and atrium, and the degree of mitral and aortic valve regurgitation were also better defined in the present study compared with multi-center studies. In future research we plan to investigate a larger cohort of patients to clarify the risk factors for dilatation or dissection of the aorta in patients with Marfan syndrome during pregnancy.

Conclusion

An increased size of the sinus of Valsalva (≥40 mm) was found in Japanese patients with Marfan syndrome who experienced aortic dilatation or dissection during or after pregnancy. The ASI (size of the sinus of Valsalva/body surface area) is a better indicator of the risk for aortic dilatation or dissection during pregnancy and after birth, compared to the absolute size of the sinus of Valsalva. Until a molecular-based approach is available to identify patients at high cardiovascular risk, echocardiographic variables will remain as the most important prognostic factors. Prospective validation of the present proposed criteria is needed, but we suggest that the present strategy may be particularly useful for treatment of women with a small physique, who are common in the Japanese population.

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Disclosure

None of the authors have a conflict of interest to disclose.

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