The Association of Endothelial Dysfunction and Cardiovascular Events in Healthy Subjects and Patients with Cardiovascular Disease*

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Abstract

Background: Endothelial dysfunction is recognized as a major factor in the development of atherosclerosis and it has a prognostic value.

Objectives: To detect the long-term association of peripheral vascular endothelial function and clinical outcome in healthy subjects and patients with cardiovascular disease.

Methods: We prospectively assessed brachial artery flow-mediated dilatation in 110 consecutive subjects (46 CVD patients and 64 healthy controls), mean age 57 ± 11 years; 68 were men. After an overnight fast and discontinuation of all medications for ≥ 12 hours, percent improvement in FMD and nitroglycerin-mediated vasodilatation were assessed using high resolution ultrasound.

Results: %FMD but not %NTG was significantly lower in CVD patients (9.5 ± 8.0% vs. 13.5 ± 8.0%, $P = 0.012$) compared to healthy controls (13.4 ± 8.0% vs. 16.7 ± 11.0%, $P = 0.084$; respectively). In addition, an inverse correlation between %FMD and the number of traditional CVD risk factors was found among all study participants ($r = -0.23$, $P = 0.015$) and healthy controls ($r = -0.23$, $P = 0.036$). In a mean follow-up of 15 ± 2 months, the composite CVD endpoints (all-cause mortality, myocardial infarction, hospitalization for heart failure or angina pectoris, stroke, coronary artery bypass grafting and percutaneous coronary interventions) were significantly more common in subjects with FMD < 6% compared to subjects with FMD > 6% (33.3% vs. 12.1%, $P < 0.03$, respectively).

Conclusions: Thus, brachial artery %FMD provides important prognostic information in addition to that derived from traditional risk factor assessment.

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The vascular endothelium is a large paracrine organ, which is the source of several secretions that regulate vessel diameter, cell proliferation, platelets and leukocyte interaction and thrombus formation. The endothelium senses and responds to many exogenic and endogenic factors via membranous cell receptors and via signal transduction mechanisms that generate and release vasoactive substances and growth factors [1]. Endothelial dysfunction is considered an important factor in atherosclerosis, hypertension and heart failure [1,2].

In the last decade, non-invasive assessment of flow-mediated dilatation of the brachial artery by ultrasound was developed [3-6]. Blood flow induces secretion of several substances including nitric oxide by the endothelium, causing vasodilatation. In addition, vessel dilatation can be measured after administration of sublingual nitrates (nitroglycerin-mediated vasodilatation). The dilatation is endothelium-independent. For the measurement of FMD and NTG, high resolution ultrasound (7.5–15 MHz) is used.

FMD is the parameter of endothelial function, and there is an inverse correlation between FMD and atherosclerosis [3]. On the other hand, NTG does not indicate endothelial function, but rather a smooth muscle function [1]. Although many risk factors are associated with the development of atherosclerosis and cardiovascular mortality and morbidity, endothelial dysfunction is the final common pathway for these factors. Endothelial dysfunction can be observed many years before the development of overt atherosclerotic anatomic lesions in the intima in patients with cardiovascular risk factors [4]. In atherosclerotic arteries, impaired endothelial function can be detected by increased vasoconstriction following various triggers and even by paradoxical vasoconstriction [7-10].

Endothelial dysfunction of coronary or peripheral arteries is an independent risk factor for cardiovascular events and provides important prognostic data in addition to the classic cardiovascular risk factors [11]. However, the effect of many classical and non-classical cardiovascular risk factors on endothelial function in healthy and cardiovascular patients is not obvious. The aim of the current study was, therefore, to investigate the association of peripheral vascular endothelial function, assessed by brachial ar-

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CVD = cardiovascular disease

%FMD = percent improvement in flow-mediated dilatation

%NTG = percent improvement in nitroglycerin-mediated vasodilatation

tery vasoreactivity, and its long-term outcome in healthy subjects compared to patients with cardiovascular disease.

Patients and Methods

Patients

We prospectively and consecutively recruited 110 consecutive patients from the Endothelial Function Assessment Laboratory of the Heart Institute at Sheba Medical Center. These included healthy subjects and stable CVD patients. Healthy subjects were defined as subjects without any history of chest pain or myocardial infarction, coronary artery bypass grafting surgery, coronary angiography with angioplasty and/or stenting, cerebrovascular accident, or peripheral vascular disease, with normal electrocardiograms and echocardiography on admission. Stable CVD patients were patients with history of myocardial infarction, CABG operation, coronary angiography with angioplasty or stenting, heart failure secondary to coronary artery disease, or CVA. Exclusion criteria included atrial fibrillation, sinus bradycardia (heart rate < 50 beats/minute) without pacemaker, sick sinus syndrome, second or third-degree atrioventricular block, intolerance to nitrates, renal failure with serum creatinine > 3 mg/dl, history of drug or alcohol abuse, chronic liver disease, or refusal to sign the informed consent form.

Following an overnight fast and the discontinuation of all medications for ≥ 12 hours, a physical examination, brachial artery reactivity testing, echocardiographic assessment, and blood tests to measure lipids, blood cell count, electrolytes, fasting glucose, homocysteine, and high sensitivity C-reactive protein, were performed. The blood samples were centrifuged immediately for 15 minutes at 3000/min. The sera were stored in -20°C. All the blood samples were tested at the end of the study in the same laboratory and by the same operators who were blinded to the patients' clinical status and endothelial function results. The hospital review board approved the study, and all participants gave written informed consent.

Vascular function protocol

The endothelial function in the form of endothelium-dependent brachial artery FMD was measured as previously described [1,12]. Briefly, FMD was assessed in the subject's right arm in the recumbent position in a temperature-controlled room (22°C) after a 10 min equilibration period, by a single ultrasonographer blinded to the patient's clinical status (healthy or CVD patient). Using a 15-6 MHz linear array (15-6L HP) ultrasound (HP SON09-09OS 5500 CV system, Agilent Technologies Inc., Andover, MA, USA), the brachial artery was longitudinally imaged approximately 5 cm proximal to the antecubital crease, where the clearest image was noted. When an acceptable image was obtained, the surface of the skin was marked, and the arm and the ultrasound probe were kept in the same position by the ultrasonographer throughout the study. An ECG was monitored continuously, and blood pressure was taken in the left arm every minute throughout the study.

- *Study phases*

a) Endothelium-dependent FMD: following a 2 min baseline period, a frozen 3 cm longitudinal image of vessel without color flow was obtained and frozen for 5 sec. The image was then unfrozen and switched to a pulsed-wave Doppler for 5 sec at a sweep speed of 50 mm/sec. A pneumatic tourniquet (Hokanson, AG101, Bellevue, WN) placed around the forearm proximal to the target artery was inflated after the baseline phase to a pressure of 50 mmHg above the subject's systolic blood pressure (or until no blood flow was noted through the brachial artery by the Doppler probe), and this pressure was held for 5 minutes. Increased flow was then induced by sudden cuff deflation. A continuous scan was performed at deflation, 60 and 90 seconds after cuff deflation, with frozen and Doppler measurements recorded at similar intervals to the baseline phase.

b) NTG-induced (non-endothelium-dependent) vasodilation: 13 minutes after cuff deflation, a second 2 min baseline-resting scan was recorded to confirm vessel recovery. After the administration of a sublingual NTG tablet (Nitrostat, 0.4 mg, Parke-Davis, NJ), scanning was performed continuously for 5 min.

- *Data analysis*

The ultrasound images were recorded on an S-VHS videotape with an SLV-RS7 videocassette recorder (Sony, CA). The diameter of the brachial artery was measured from the anterior to the posterior interface between the media and adventitia ("m line") at a fixed distance. The mean diameter was calculated from four cardiac cycles synchronized with the R-wave peaks on the ECG. All measurements were recorded at end-diastole to avoid possible errors resulting from variable arterial compliance. The internal diameter was calculated with PC Prosound software (USC, Los Angeles) using a Horita Data Translation Image Processing board (DT2862-60Hz; Mission Viejo, CA). The diameter percent change caused by endothelium-dependent flow-mediated vasodilatation and endothelium-independent percent change from baseline in NTG-mediated vasodilatation were expressed as the percent change relative to that at the initial resting scan. The intra-observer correlation coefficient for baseline and deflation diameters was 0.99. The absolute error between measurements ranged from 0 to 0.12 mm (for brachial artery diameter) and 0.02% to 2.98% (for %FMD).

- *Echocardiographic assessment*

All patients underwent two-dimensional echocardiographic assessment within 30 days of the endothelial function assessment by the same senior cardiologist (M.S.F.) who was blinded to the patients' clinical status and endothelial function results. Echocardiographic data included left ventricular dimensions and volumes, left atrial dimension and volume (biplane modified Simpson's), LV ejection fraction (biplane

CABG = coronary artery bypass graft
CVA = cerebrovascular accident

LV = left ventricular

modified Simpson's), LV filling pattern, the degree of mitral regurgitation, and assessment of the myocardial E-wave by tissue Doppler imaging of the LV mean annular septal and lateral walls. Mitral inflow was assessed with pulsed-wave Doppler echocardiography from the apical four-chamber view. The Doppler beam was aligned parallel to the direction of flow, and the sample volume was placed between the tips of mitral leaflets. From the mitral inflow profile, the diastolic E- and A-wave peak velocities and the E-wave deceleration time were measured [13].

- *Long-term follow-up*
  All patients were followed by phone for 15 ± 2 months, for combined CVD endpoints, including all-cause mortality, myocardial infarction, hospitalization for heart failure or angina pectoris, CVA, CABG and percutaneous coronary interventions by physicians who were blinded to the patients' baseline clinical status and endothelial function results. All telephonic data received from the patients were compared to the hospital records.

Statistical analysis

All the clinical and laboratory data were summarized in statistical software SPSS (version 11). Chi-square test was utilized for categorical variants, *t*-test for comparison of two groups, and linear regression for continuous variables. Multivariate analysis was performed. Statistical significance was determined when $P < 0.05$.

Results

The study population comprised 110 subjects – 68 (62%) men and 42 (38%) women – with a mean age of 57 ± 11 years (range 17–81); 46 of the patients (42%) had CVD and 64 were healthy controls (58%).

The healthy control group included 43 subjects with no history of chest pain, 9 with history of non-specific chest pain, and 12 subjects with chest pain and angiographically normal coronary arteries. The patient group included 15 patients with coronary artery disease, 6 with CVA, and 25 patients with CVD and congestive heart failure, CVA or intractable angina pectoris. The clinical and laboratory data for the patient and healthy groups are summarized in Tables 1 and 2.

Endothelial function in healthy and CVD patients

The baseline brachial artery diameter was significantly greater in the patient group compared to the healthy group [Table 1] and in men compared to women (6.24 ± 1.00 vs. 4.85 ± 0.70 mm, $P = 0.0001$). %FMD but not %NTG was significantly lower in CVD patients compared to healthy subjects (9.5 ± 8.0% vs. 13.5 ± 8.0%, $P = 0.012$, and 13.4 ± 8.0% vs. 16.7 ± 11.0%, $P = 0.084$, respectively) [Table 1]. In healthy subjects %FMD was almost the same in those with or without chest pain (13 ± 6% vs. 14 ± 9%, $P = 0.807$, respectively). CVD patients with CHF, CVA and/or intractable angina pectoris (n=25) had a non-significant lower %FMD compared to those with CVD only (n=15) (8 ± 9% vs. 13 ± 7%, $P = 0.079$, respectively).

CHF = congestive heart failure

Table 1. Baseline characteristics of study participants

	Healthy subjects (n=64)	Patients (n=46)	*P*
Age (yrs)	55 ± 11	60 ± 12	0.023
Body mass index (kg/m^2)	27 ± 4	27 ± 3	0.936
Total cholesterol (mg/dl)	209 ± 35	179 ± 39	0.001
LDL-C (mg/dl)	132 ± 28	107 ± 29	0.001
Triglecyrides (mg/dl)	123 ± 71	160 ± 96	0.023
HDL-C (mg/dl)	52 ± 14	41 ± 9	0.000
Homocysteine (µmole/L)	13 ± 3	14 ± 6	0.065
hs-CRP (mg/L)	4 ± 7	5 ± 10	0.716
Glucose (mg/dl)	95 ± 21	120 ± 52	0.002
Systolic blood pressure (mmHg)	137 ± 17	140 ± 23	0.480
Diastolic blood pressure (mmHg)	79 ± 8	79 ± 10	0.773
Pulse pressure (mmHg)	58 ± 13	61 ± 20	0.305
Mean arterial pressure (mmHg)	99 ± 10	99 ± 13	0.797
Left ventricular ejection fraction (%)	61 ± 8	54±16	0.003
Baseline brachial artery (mm)	5.43 ± 1.00	6.11 ± 1.00	0.001
%FMD	13.4 ± 8.0	9.5 ± 8.0	0.012
%NTG	16.7 ± 11.0	13.5 ± 8.0	0.084

Values are expressed as mean ± SD
%FMD, %NTG = % change from baseline in brachial artery diameter is caused by FMD and NTG, respectively. LDL = low density lipoprotein.

Table 2. Cardiovascular risk factors and concomitant medications

	Healthy (n=64)	Patients (n=46)	*P*
Females	(53%) 34	(17%) 8	0.001
Males	(47%) 30	(83%) 38	
Hypertension	(36%) 23	(48%) 22	0.242
Hyperlipidemia	(47%) 30	(54%) 25	0.562
Smoking	(14%) 9	(17%) 8	0.790
Diabetes mellitus	(6%) 4	(24%) 11	0.011
Family history of coronary artery disease	(56%) 36	(35%) 16	0.034
Medications			
Aspirin	(39%) 25	(57%) 26	0.083
Statin	(31%) 20	(59%) 27	0.006
Calcium channel blockers	(13%) 8	(13%) 6	1
Diuretics	(11%) 7	(28%) 13	0.025
Angiotensin-converting enzyme inhibitors	(20%) 13	(33%) 15	0.184
Beta-blockers	(16%) 10	(41%) 19	0.004

Correlation of %FMD with CVD risk factors

The correlation of %FMD with CVD risk factors is summarized in Table 3. %FMD was significantly lower in men compared to women (10.4 ± 8.0% vs. 14.0 ± 9.0%, $P = 0.024$). Age was inversely correlated with %FMD ($r = -0.228$, $P = 0.017$). %FMD was

lower in diabetic compared to non-diabetic patients, however, without statistical significance (8.3 ± 8.0% vs. 12.3 ± 8.0%, P = 0.082). No correlation was found between history of hypertension, hyperlipidemia, family history of CVD, or smoking and %FMD. Subjects treated with aspirin (10.1 ± 7.0% vs. 13.2 ± 9.0%, P = 0.049) and angiotensin-converting enzyme inhibitors (9.2 ± 8.0% vs. 12.7 ± 8.0%, P = 0.053) had significantly lower %FMD compared to those not treated. In addition, no correlation was found between statins, calcium channel blockers, diuretics, or beta-blocker therapy and %FMD.

Nevertheless, high density lipoprotein cholesterol was significantly and positively associated with %FMD. No correlation was found between body mass index, total cholesterol, low density lipoprotein cholesterol, triglycerides, fasting blood glucose, or homocysteine and %FMD [Table 3]. In addition, there was no correlation between systolic, diastolic and mean arterial blood pressure and %FMD. However, there was a non-significant inverse correlation between pulse pressure and %FMD. In addition, there was no correlation between left ventricular ejection fraction and %FMD in all study groups. Multivariate analysis indicated age to be the most significant factor affecting %FMD (P < 0.011).

%FMD and the number of CVD risk factors

The total number of classical CVD risk factors (including male gender, age (males > 55 years old, females > 65 years old), family history of premature CVD, hypertension, hyperlipidemia, diabetes mellitus, obesity (BMI > 25 kg/m^2) was inversely and significantly associated with %FMD (r = -0.23, P = 0.015) for all the study participants (healthy and patients). The same trend was found when only the healthy group was analyzed (r = -0.23, P = 0.036).

%FMD and baseline brachial artery diameter

Among all study participants (healthy and patients), a significant inverse correlation was observed between baseline brachial artery diameter and %FMD (r = -0.51, P = 0.000).

Table 3. Correlation of %FMD and cardiovascular risk factors

	%FMD	
	r	*P*
Age	-0.228	0.017
Body mass index	-0.139	0.146
Total cholesterol	0.074	0.444
Low density lipoprotein cholesterol	0.035	0.722
High density lipoprotein cholesterol	0.252	0.008
Homocysteine	0.037	0.704
Glucose	-0.076	0.473
High sensitivity C-reactive protein	-0.064	0.509
Triglycerides	-0.126	0.190
Systolic blood pressure	-0.138	0.150
Diastolic blood pressure	-0.15	0.88
Pulse pressure	-0.160	0.094
Mean arterial pressure	-0.089	0.357

Table 4. Composite* cardiovascular endpoint in a mean follow-up of 15 ± 2 months

	FMD < 6%	FMD > 6%	*P*
All subjects (n=109)			
With composite endpoint	9/27 (33.3%)	10/82 (12.1%)	< 0.03
Without composite endpoint	18/27 (66.7%)	72/82 (87.9%)	
Patients (n=45)			
With composite endpoint	8/15 (53.3%)	8/30 (26.7%)	< 0.1
Without composite endpoint	7/15 (46.7%)	22/30 (73.3%)	

* Composite endpoint including all-cause mortality, myocardial infarction, hospitalization for heart failure or angina pectoris, stroke, CABG and percutaneous coronary interventions.

Composite clinical CVD endpoints

In a mean 15 ± 2 month follow-up of 109/110 study participants (99%), the composite CVD endpoints were significantly more common in subjects with FMD < 6% than in those with FMD > 6% (33.3% vs. 12.1%, P < 0.03, respectively) [Table 4]. In addition, in CVD patients there was only a trend towards more composite endpoints among those with FMD < 6% compared to those with FMD > 6% (53.3% vs. 26.7%, P < 0.100).

Discussion

Our study demonstrates that peripheral vascular endothelial function, assessed by brachial artery %FMD, is significantly impaired in CVD patients whose baseline brachial artery diameter is also significantly higher, compared to healthy non-CVD subjects. In addition, increasing baseline brachial artery diameter is correlated with endothelial dysfunction. These results concur with those of Celermajer et al. [14] who found that reduced %FMD was related to larger vessel size in clinically healthy subjects. We also found an inverse correlation between the total number of classical CVD risk factors and endothelial dysfunction in all study participants and especially in healthy volunteers, which reinforces Celermajer's results in healthy subjects [14].

Our finding that endothelial function is significantly impaired in the CVD patient group also concurs with previous studies [1-4,15], while the %NTG was not significantly impaired in these patients. This result is similar to other reports that noted the impact of risk factors such as diabetes on smooth muscle function and could potentially implicate abnormalities in guanylate cyclase-cyclic guanosine monophosphate signaling pathways to low perfusion states seen in patients with advanced CHF and intractable angina pectoris [16]. Although most studies reported a non-significant effect of wellness on %NTG, there is evidence that CVD risk factors may impair it [17].

Although some reports demonstrated impaired coronary endothelial function in subjects with chest pain and non-significant CVD [18], we did not observe any difference in %FMD, neither in asymptomatic healthy subjects nor in healthy subjects with chest pain. This discrepancy may be partially explained by the small number of healthy subjects in the current study and the concomitant medications which could beneficially improve

BMI = body mass index

vascular endothelial function, such as aspirin, statins and ACE inhibitors [19].

Endothelial dysfunction is a marker and barometer of atherosclerotic risk and is usually impaired in advanced CVD [15,19,20]. In the current study endothelial dysfunction was non-significantly worse in patients with advanced CVD (i.e., CVD patients with CHF and intractable angina pectoris) compared to stable CVD patients ($P = 0.079$). The lack of significance may be due to the small number of patients. Endothelial function was found to be a negative predictive parameter in CVD patients [11,19].

Endothelial function was affected by gender and age: male gender and older age were associated with lower endothelial function and CVD. However, endothelial function was not associated with hypertension, hyperlipidemia, family history, diabetes or smoking, which could be due to the relatively small number of subjects. Surprisingly, subjects treated with aspirin and ACE inhibitors also had lower endothelial function compared to those without such treatment [19]. One explanation might be that subjects treated with aspirin and ACE inhibitors had relatively more CVD risk factors than non-treated subjects, or those patients had advanced CVD with CHF.

Our current study demonstrated the correlation between HDL-cholesterol and endothelial function. HDL-cholesterol is a protective CVD risk factor associated with enhanced endothelial function. However, hyperlipidemic subjects with low HDL-cholesterol and without overt CVD have impaired endothelial function due to lack of inhibition of oxidation and over-expression of adhesion molecules [21].

No correlation was found in the current study between homocysteine and endothelial function, although homocysteine is considered a novel CVD risk factor and was already associated with endothelial dysfunction [22]. One explanation may be that the serum levels of homocysteine in the current study participants (healthy and CVD patients) were within the normal range. In addition, we could not demonstrate any association of hs-CRP, an inflammatory parameter associated with atherosclerosis [23], and endothelial function. As in the case of homocysteine, the levels of hs-CRP were also within the normal range in healthy and CVD patients. Additionally, hs-CRP is usually affected by statin and antiplatelet therapy, weight reduction and physical activity [24]. These treatments might partially explain the lack of association between endothelial function and hs-CRP observed in the current study.

Multivariate analysis showed that age was the single independent factor that was inversely associated with endothelial function. Increasing age causes damage to the vessel wall from structural and functional changes occurring in the extracellular matrix, smooth muscle cell and the endothelial layer of vascular vessels [25]. In addition, there is disequilibrium between the vasodilator and vasoconstrictor factors released by the endothelium [25]. This process occurs during aging, independently of other risk factors, and explains the increase in cardiovascular events in the elderly.

In addition, we found a significantly inverse correlation between the number of classical CVD risk factors and endothelial function among all the study subjects and among the healthy group. These data support the hypothesis that endothelial function is the final common pathway of all traditional CVD risk factors, and the magnitude of endothelial dysfunction is due to the summation of all CVD risk factors [15,18,20].

An interesting finding was the inverse association of the baseline brachial artery diameter and endothelial function. This finding suggests that subjects with lower baseline brachial artery diameter may have higher %FMD than those with a higher diameter. A possible explanation is that an artery with a large diameter does not have the potential of maximal dilatation as does an artery with smaller diameter. This finding was previously reported by our group and is concordant with the observation that endothelial function is usually better in women than in men, where the baseline brachial artery diameter among women is smaller than in men, as observed in the current study and others [1].

Our most striking finding is the association between low brachial artery endothelial function and long-term CVD clinical outcome. This finding corresponds with previous studies demonstrating the impact of brachial artery FMD on clinical outcome in healthy subjects and CVD patients [11,15,19,20,23].

Study limitations

Our study group comprised a relatively small number of healthy subjects and stable CVD patients with near-optimal lipid values and markers of inflammation. Further studies with larger numbers of healthy subjects and CVD patients are indicated, given these results.

There is both biological and measurement variability in the ultrasound assessment of brachial artery FMD. However, prior work has demonstrated the feasibility of this approach, which if performed carefully, can detect change in relatively small sample sizes [1].

Conclusions

Assessment of brachial artery %FMD provides important prognostic information in addition to that derived from traditional CVD risk factor assessment in humans. Larger long-term studies are required to determine the clinical value of these results.

ACE = angiotensin-converting enzyme
HDL = high density lipoprotein
hs-CRP = high sensitivity C-reactive protein

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Capsule

Pregnancy after breast cancer

Ives et al. tried to identify women who survived breast cancer and subsequently conceived and to determine the rate of pregnancy (proportion), management, outcome of the cancer, and outcome of the first subsequent pregnancy. The design was a population-based descriptive study with cases identified from the Western Australian data linkage system and validated by review of medical charts. Supplementary data were obtained from hospital and clinician records. The participants were women aged < 45 with a diagnosis of breast cancer who subsequently conceived. The results showed that 62 women (54%) with a diagnosis of breast cancer who subsequently conceived did so less than 2 years after their diagnosis: 29 of them had an abortion, 27 had a live birth, and six miscarried. Within a proportional hazards regression model subsequent pregnancy was associated with improved overall survival (hazard ratio 0.59, 95% confidence interval 0.37–0.95). When the model was stratified by time from diagnosis subsequent pregnancy was associated with improved overall survival in women who waited at least 24 months to conceive (0.48, 0.27 to 0.83) and a non-significant protective effect was seen for women who waited at least 6 months to become pregnant. The authors conclude that this study does not support the current medical advice given to premenopausal women with a diagnosis of breast cancer to wait 2 years before attempting to conceive. This recommendation may be valid for women who are receiving treatment or have systemic disease at diagnosis, but for women with localized disease early conception, 6 months after completing their treatment, is unlikely to reduce survival.

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